1. Introduction

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Bovine spongiform encephalopathy (BSE) was first recognized and defined as a pathological entity in the United Kingdom in November 1986 (Wells et al., 1996). Initial epidemiological investigations and examination of archived brains indicated that the first cases occurred around April 1985. Subsequently, the largest freeborn epidemic of a transmissible spongiform encephalopathy (TSE) occurred which has had severe economic effects and has caused concern for public
health.

The initial clinical signs of this fatal neurological disease are non-specific, typically involving behavioural changes. As the clinical phase progresses the disease is typified by alterations in mental state and of sensation and ataxia. The majority of affected animals reach the advanced stages within two to three months from the onset, when slaughter on welfare grounds becomes necessary (Wilesmith et al., 1988).

The disease affects adult animals, with a peak age at onset of four to five years of age. Both sexes and all breeds are equally susceptible.

BSE can only be confirmed post-mortem by pathological examination of brain tissue. Histological examination of the hind brain has been the primary method for the statutory diagnosis in the United Kingdom (Wells et al., 1989). The histological changes are typical of the TSEs. Microscopic lesions in the central nervous system consist of bilaterally symmetrical, non inflammatory vacuolation of neuronal perikarya and grey matter neuropil.

BSE is transmissible by parenteral inoculation of inbred strains of mice (Fraser et al., 1988) and cattle (Dawson, Wells and Parker, 1990). In cattle the incubation period is approximately 18 months using brain homogenates from natural terminal cases.

Epidemiological studies identified the vehicle of infection to be meat and bone meal incorporated as a protein source in concentrated feedstuffs (Wilesmith et al., 1988). The most probable origin of BSE was sheep scrapie. Most BSE cases forming the epidemic resulted from the recycling of infected cattle tissues, via meat and bone meal, within the cattle population (Wilesmith, Ryan and Atkinson, 1991; Wilesmith and Wells, 1991). A ban on the feeding of ruminant protein to ruminants was introduced in July 1988 (HMSO, 1988b), shortly after the disease was made statutorily notifiable in June 1988. This feed ban was effective such that the incidence began to decline in 1993. However, it was not completely effective essentially because of a problem of cross-contamination of cattle feedstuffs with ruminant protein, notably during its production (Wilesmith, 1996a; 1996b). Therefore the feeding of mammalian protein to all farm animal species was prohibited in April 1996 (HMSO, 1996). The cross-contamination problem highlighted the low dose exposure phenomenon in the epidemiology of BSE. The low-dose exposure phenomenon had been recognized early in the course of the epidemic (Kimberlin and Wilesmith, 1994) and has subsequently been confirmed by studies of experimental oral exposure of cattle. In
these studies 1 g of brain, wet weight, from a terminal case resulted in disease with an incubation period within the range observed naturally (G.A.H. Wells and A.R. Austin, personal communication).

The occurrence of BSE has had a major impact on the trade in cattle, beef and bovine products from the United Kingdom. There have been consequences on a wide range of industries throughout the world, notably the production of pharmaceutical products. It is likely that BSE has resulted in the occurrence of feline spongiform encephalopathy (FSE) in domestic cats and cases of spongiform encephalopathy in exotic species bred in zoological collections in the United Kingdom. More important has been the public health concern. In November 1989 the use of certain specified bovine offals (SB0s), which potentially contain relatively high titres of BSE infectivity, were banned for the use in human food. Subsequent studies of the pathogenesis of BSE have indicated that these were appropriate tissues to exclude. However, in March 1996 a new variant of the human TSE, Creutzfeldt-Jakob disease (CJD), was identified in the United Kingdom. Strain typing studies in inbred strains of mice of this new form of CJD (\(v_CJD\)) revealed similar patterns to those seen in BSE (Bruce et al., 1997). The identification of these patterns has raised questions about whether there will be a major epidemic of \(v_CJD\) (Cousens et al., 1997). However, the BSE epidemic in the United Kingdom has continued to decline. Although there may be a slightly enhanced risk of infection for offspring of clinical cases, at least while there was potential exposure from the feedborne source, there is no evidence for a natural transmission of BSE between cattle sufficient to prolong the epidemic, and it is clear that the incidence of BSE in the early part of the next millennium will be very low.

OTHER TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSEs)

A number of TSEs had been identified and defined before the occurrence of BSE. They all share similar pathological and therefore clinical features, but there are a number of differences between them. The following summarizes the essential features of these related diseases.

**Scrapie**

Scrapie in sheep is the original TSE and has been an endemic disease of sheep in the United Kingdom for over 200 years. It has been recorded in many other countries of the world, with Australia and New Zealand being notable exceptions. The disease also occurs
in goats, but scrapie in this species has not been subject to the same degree of research.

Scrapie in sheep has been the subject of research both as a natural disease and in laboratory studies using established inbred strains of mice. Laboratory studies provided the basis for the elucidation of the genetic risk factor in sheep scrapie. However, without the modern molecular genetic techniques, epidemiological studies of natural sheep scrapie were difficult, as the confounding from this source could not be appropriately accounted for. Therefore, although there were a relatively large number of studies of natural sheep scrapie in the 1950s and 1960s, their interpretation is difficult (Hoinville, 1996). The more recently established molecular genetic techniques have allowed the identification of three major polymorphisms of the PrP gene of sheep which have some importance in the control of the susceptibility of sheep to scrapie. Differences between breeds in the importance of each of these polymorphisms have emerged from the molecular genetic research in recent years (reviewed in Hunter et al., 1994).

There is some hope that the genotyping of sheep will assist in the control of sheep scrapie and replace the previously employed control method of culling female and male lines in scrapie-affected flocks. Culling required sound identification of sheep and good record keeping and was based not just on the likely inheritance of the then unknown polymorphisms of the PrP gene, but on the fact that offspring of scrapie-affected ewes had a greater risk of developing disease. The apparently increased maternal risk factor probably results from the fact that the scrapie agent has only been detected in the foetal membranes of affected sheep, and not in any other excretions or secretions. However, there is evidence that horizontal transmission occurs from the same source. A review and synthesis of the results of past research on sheep scrapie indicates that, in high incidence flocks at least, horizontal transmission of scrapie is of equal importance to maternal transmission (Hoinville, 1996).

Scrapie is important as it is the most probable origin of BSE. However, the genetic risk factors are absent in BSE (Hunter et al., 1994) and it is highly unlikely that BSE will be naturally sustained in the cattle population because of the absence of any evidence of horizontal transmission. Extensive epidemiological studies have failed to reveal an epidemiological association between scrapie and CJD. One example is the more or less uniform incidence of CM in countries throughout the world and the varying occurrence and presence of scrapie in the national sheep populations.
Transmissible mink encephalopathy (TME)

Transmissible mink encephalopathy (TME) is a very rare disease of ranch-reared mink, but when it occurs, the mortality rate is high and can be 100 percent of the breeding animals. TME has been reported in North America and a number of European countries, but not in the United Kingdom, perhaps because fanned mink in the United Kingdom have been fed on commercially produced concentrates rather than abattoir waste or dead animals, which have been incriminated as the source of infection for mink.

As in BSE, the evidence indicates that sheep scrapie is the origin of TME, and mink are a dead-end host, unless there is cannibalism. There are therefore differences between scrapie and TME. It is interesting to note that there have been no recent TME outbreaks in North America, perhaps because mink ranchers have been made aware of the risks of feeding mink with the carcasses of sheep which may or may not have died from or have been suffering from scrapie.

Kuru

Kuru was the main cause of death in the localized Fore-speaking tribe in Papua New Guinea. The origins and dynamics of the kuru epidemic have some similarities to those of the BSE epidemic. Unfortunately records of the incidence of kuru in the early years do not exist. It seems possible that this disease originated from a spontaneous case or cases of CJD, and kuru occurred as a result of ritual cannibalism of dead relatives. Therefore the kuru agent was "passaged" within families much in the same way as BSE was recycled in cattle tissues via meat and bone meal. The cannibalistic activities declined in the late 1950s and the kuru epidemic is clearly nearing its end. Humans are a dead-end host for the disease.

Creutzfeldt-Jakob disease

Two basic forms of TSE occur in humans. Gerstmann-Strdussler syndrome (GSS) has a genetic basis and manifests as an autosomal dominant disease. It is classically associated with the codon 102 Pro-Leu change. CJD occurs in a sporadic form, which accounts for 85 percent of cases, and as a familial form. In the latter case there are now known to be several point mutations and expansions in an octapeptide repeat sequence within the PrP gene open reading frame associated with the disease (Poulter et al., 1992).

The sporadic disease is very rare and remarkably uniform throughout the world, affecting approximately one in a million people.
Since CJD was first known to be transmissible in 1968, the possibility that the disease was associated with scrapie has been studied widely and intensively, but with no supporting evidence.

A new variant of CJD (nvCJD) was identified in March 1996 in the United Kingdom. It was classified as a new variant because of differences in the histological changes in the brain, because of clinical signs and because to date (October 1997) it has predominantly affected people less than 40 years of age. So far 22 cases have occurred in the United Kingdom and one case in France, where BSE has also occurred, but at low incidence (see Chapter 4). Its occurrence raised the level of concern that the BSE agent was responsible. The initial results of strain typing of nvCJD in inbred strains of mice have provided evidence that agents of BSE and nvCJD are very similar and BSE is therefore the likely source (Bruce et al., 1997). Current research is consequently directed at determining the ultimate size of the epidemic and how humans may have become exposed. Research has also stimulated an interest in potential therapeutic strategies and means of diagnosis of the infection in its pre-clinical stage.

**The nature of the infectious agent responsible for the TSEs**

The nature, and more specifically the molecular structure, of the infectious agent of the TSEs has been the subject of much scientific debate and at times controversy, but at present it remains unknown. An accepted aspect is that one important component of the disease-inducing agent is a post-translational form of the normally produced PrP protein. This normal form is conventionally abbreviated to PrPc, the superscript "C" standing for the normal cellular form. The abnormal form associated with disease is, perhaps unfortunately, referred to under a number of abbreviations: PrP'l, PrP's' and PrP'RE' - the superscripts referring to scrapie, BSE and resistance to enzymatic, proteinase-K digestion respectively. The last of these characteristics is acknowledged as a valid discriminatory test between the normal and abnormal forms of the PrP protein.

There are two basic hypotheses as to the nature of the infectious agent associated with TSEs. One is that the infectious agent consists of only the modified form of the normal cellular protein, whose function is still not known. This hypothesis was originally outlined by LS. Griffiths and later adopted by Stanley Prusiner, who in 1982 achieved biochemical purification of the scrapie agent. This he termed a "prion", equivalent to a "prion protein", which was considered to be the major component of the infectious agent.
The other main hypothesis is that the infectious agent contains an information molecule, if not a nucleic acid. There are two subhypotheses within this, one being the virus model and the other a virino model. The adherence to these hypotheses is mainly a result of the acknowledged occurrence of strains of the TSE agents, as determined by transmission to specific inbred strains of mice.

The research and debate on the nature of the TSE agent will undoubtedly continue for a few more years. Supporters of the virus and virino hypotheses await the finding of a nucleic acid or a component that has a similar function. Proponents of the protein-only hypothesis are seeking evidence to explain how strain variation and mutation can be based on a post-translationally modified, normal protein.

2. Epidemiology

BSE was first recognized in the United Kingdom in 1986 as a result of the routine animal disease surveillance activities. The initial epidemiological studies and histological examination of archived bovine brains indicated that the first cases occurred around 1985. The detection of BSE was probably aided by the relatively high degree of active communication between animal keepers and veterinarians, who in turn seek help from the network of Veterinary Investigation Centres, whose staff may seek specialist advice from the Central Veterinary Laboratory. In a more practical sense the disease was identified by its unusual clinical presentation, by the fact that one of the earliest affected herds was very large and multiple cases occurred, and by talk among herd owners about their "unusual" animals, which stimulated the owners to seek veterinary attention (Wilesmith, 1996a). Although only a small number of cases had been confirmed by histological examination by early 1987 (Wells et al., 1992), the epidemiological study primarily to investigate the potential aetiologies was started in June 1987.

Early studies on the possible aetiology:

The early studies relied on making veterinarians involved in cattle medicine aware of the clinical signs and requesting their voluntary notification. Although the pathological features were reminiscent of scrapie, other potential aetiologies were not excluded. Vehicles of a scrapie-like agent included vaccines, hormones and other biological products, direct or indirect contact with sheep and a variety of free-living animals, imported cattle and semen and feedstuffs containing animal-derived products. The other main potential aetiology was a
toxic phenomenon resulting from the use of agricultural chemicals, such as herbicides and pesticides, and pharmaceutical products including organophosphorus preparations, synthetic pyrethroids and anthelmintics. A solely genetic origin was also investigated.

All of the possible vehicles, except feedstuffs, and the other actiologies, were eliminated by December 1987 following the completion of a case study of nearly 200 affected herds and cases of BSE (Wilesmith et al., 1988). With respect to feedstuffs, two possible vehicles of infection were evident: meat and bone meal and tallow. The balance of evidence showed that meat and bone meal was the primary vehicle and therefore responsible for most, if not all, cases.

The suggestion that meat and bone meal was the primary vehicle was based initially on a consideration of the physicochemical properties of the scrapie agent which make it more likely to partition with the protein fraction rather than the lipids of tallow. The geographical variation in incidence and the geographical distribution and handling of tallow compared with meat and bone meal supported the latter. In the early stages of the epidemic, although BSE occurred simultaneously throughout the United Kingdom, the incidence was markedly greater in the south of England (see Figures 5 and 6). Unlike meat and bone meal which, like proprietary feedstuffs, has a relatively local distribution, tallow is purchased and mixed by a relatively small number of companies which are much more distant and which distribute the product nationally. A similar incidence across the country would therefore have been expected if tallow was the primary vehicle of infection. The reasons for the north-south difference in incidence were identified in a subsequent stage of the epidemiological study and are described later.

The feedborne hypothesis in general was supported by particular features of the epidemic. The incidence of BSE is considerably greater in dairy herds than in beef suckler herds. Commercial feedstuffs containing meat and bone meal are less frequently used to feed beef suckler herds than other animals. In addition, the risk of a herd experiencing a case of BSE increased with increasing herd size. This was consistent with the fact that the larger the herd the more feed is required and the greater the chances of buying an infected batch of feed. In order to investigate the meat and bone meal hypothesis more formally, a case-control study was initiated in early 1988 by recruiting unaffected herds for potential controls, before the hypothesis had become widely known. The final analysis of this study was completed in 1991, allowing for the long incubation period, and the results provided supporting evidence that meat and
bone meal was the source (Wilesmith, Ryan and Hueston, 1992).

The initial findings were considered sufficiently strong to make suspicion of the disease statutorily notifiable in June 1988 and ban the feeding of ruminant protein to all ruminants in July 1988 (HMSO, 1988a; 1988b). Subsequently, a standard epidemiological questionnaire has been completed for all suspect cases of BSE reported and the brain of every animal slaughtered has been examined histologically. These measures have resulted in a large and valuable epidemiological database which has facilitated a detailed monitoring of the epidemic.

EXPOSURE OF THE CATTLE POPULATION

The initial epidemiological studies indicated clearly that BSE was a new disease and it had been identified very shortly after its first occurrence. Some important questions were raised by these studies: When did exposure start? Which age groups of animals were at most risk from infection? And how did the cattle population of the United Kingdom become exposed to a scrapie-like agent?

The first two questions were addressed by simulation studies (Wilesmith et al., 1988). These provided some evidence that the majority of BSE cases observed resulted from infection in calf hood, suggesting an age-dependent susceptibility, and that effective exposure of the cattle population commenced quite suddenly in 1981/82. The third question was addressed by discussions with representatives from the animal feedstuffs industry. The primary issue of exposure was whether or not there had been an increase in the inclusion rate of meat and bone meal in cattle rations during the late 1970s and early 1980s. This proved not to be the case as meat and bone meal had not recently been incorporated into proprietary cattle feedstuffs.

Attention therefore turned to the processes used for the rendering of animal waste to produce meat and bone meal. Two major changes in the industrial processes used in the United Kingdom were identified as possibly significant. The first was a change from batch processing to continuous processing. The second was a reduction in the use of hydrocarbon solvents to maximize the extraction of tallow. Other considerations included the possible risks of rendering of tissues from other species, notably mink and whales.

In order to obtain more documentary information, and therefore detailed aspects of these changes, a survey of all rendering plants in operation in the United Kingdom (excluding Northern Ireland) was
conducted in 1988 (Wilesmith, Ryan and Atkinson, 1991). In addition to investigating the details and timing of these two basic changes, the survey had the objectives of determining whether or not any rendering process was likely to have achieved a time/temperature combination capable of inactivating a scrapie-like agent and determining whether or not there had been a concentration in the rendering of abattoir waste from the slaughter of sheep.

The results of the survey confirmed two major changes in the rendering processes, but indicated that there had been no other relevant change, such as the concentration of the rendering of sheep tissues; the majority of rendering plants continued to process a mixture of tissues from the farm animal species. The change from batch processing to continuous processes was not likely to be significant in the aetiology of BSE, first because the change was a gradual one, from 1972 to 1988, and second because the survey did not reveal a difference in the mean maximum temperatures achieved in continuous and batch processes. The other change, a reduction in the use of hydrocarbon solvents, was, however, quite abrupt and coincided with the predicted start of exposure of the cattle population in 1981-1982.

The reduction in the use of hydrocarbon solvents had a geographical variation. The process was abandoned entirely in England and Wales, but remained in use in Scotland; approximately 80 percent of the meat and bone meal produced there had received this treatment. This finding provided the first explanation for the variation in the geographical variation in risk. The results of the survey of rendering plants also revealed a geographical variation in the proportion of meat and bone meal that had been produced as a result of a double heat treatment which was negatively correlated with the incidence of BSE (Wilesmith, Ryan and Atkinson, 1991).

However, it was important to establish whether this change was a biologically plausible explanation. On examination, the move away from solvent extraction would have resulted in the loss of two partial scrapie-inactivation steps. First, solvent extraction entailed, as a terminal process, an additional heat treatment for some eight hours at 70°C which would have reduced infectivity and/or made the infectivity more heat sensitive. The second step was the application of superheated steam to the meat and bone meal for 15 to 30 minutes to remove the solvent. Wet heat is much more effective against the scrapie agent than dry heat. Wet heat was also applied to a material which had been defatted to a 1 percent lipid content, and as with other conventional pathogens the presence of fat affects the thermal tolerance of the scrapie agent. It was therefore
concluded that the cessation of solvent extraction was a major factor resulting in BSE.

It follows that the cattle population had been potentially at risk for some time. A number of critics have attempted to suggest that the change in the rendering processes was the result of a deregulation of statutory powers over the rendering industry. This is not true; the reasons for the change were mainly economic. The price of tallow per tonne fell to that of meat and bone meal and the solvent extraction process was an energy intensive process. In addition, the energy content of animal feedstuffs provided by tallow was increasing. Maximizing the extraction of tallow from meat and bone meal was therefore not logical with tallow being added if meat and bone meal had a low fat content.

**DESCRIPTIVE EPIDEMIOLOGICAL FEATURES OF BSE**

The continual detailed monitoring of the epidemic in which a standard epidemiological questionnaire has been completed for every suspect case reported has allowed the descriptive epidemiological features to be subjected to more or less constant scrutiny. The following provides a summary of these features.

**National incidence and the epidemic in general**

Figure 1 shows the very basic data relevant to the epidemic - the number of suspect cases reported each week. It should be noted that it includes the suspect cases that prove not to be BSE following histological examination.

Although this presentation of data can and h been updated at weekly intervals, some care in interpretation is required. There is no constant interval between the date of official notification of a suspect case and the onset of clinical signs. addition, the proportion of suspect cases that a confirmed as cases has changed over time, with the negative rate increasing as the epidemic declines (Figure 2).

There is also an underlying seasonality in the negative rate. This is likely to be a result of the confusion between metabolic diseases which are more prevalent in the spring months, following turnout to grass usually in May each year. T1 secular increase in the negative rate does mean that the epidemiological interpretation of basic data relating to the number of suspect cases reported each week is difficult.
FIGURE 1
Number of suspect cases of BSE reported each week in the United Kingdom (excluding Northern Ireland) (June 1998 to October 1998)
The more appropriate data presentation is the epidemic curve of cases using the date of clinical onset, rather than the date reported (Figure 3).

Figure 3 indicates the peak of the epidemic in the early months of 1993. If this peak monthly incidence had been maintained for 12 months, it would have represented an annual incidence of 1 percent of adult animals. Subsequently the epidemic declined, as expected as a result of the ruminant feed ban. A notable aspect of the epidemic, as indicated by the accumulated data, is that in the absence of the feed ban the number of suspect cases reported per week could have reached 5,000 by the end of 1995. This incidence would have presented practical difficulties in the veterinary examination, in incinerating the carcasses or in maintaining data entry, compounded by associated severe financial implications.

In addition to the problem of not being able to provide a current epidemiological curve because a proportion of suspect cases being reported today will be attributed to a date of onset six months previously, this presentation does not remove the potential observational bias. There is an observed seasonality in incidence, with an increase commencing in mid-August each year. This is coincident with the change in the degree and frequency of observation of cattle related to the seasonality of calving; in the important years of the epidemic, 70 percent of cows calved in August, September and October.
An important aspect of the epidemiology of BSE is that the majority of cases that have occurred result from infection from the recycling of infected cattle tissues, via meat and bone meal. This is of crucial importance for the risk assessment for the occurrence of BSE in any country which has had BSE seeded into the cattle population as a result of the importation of either meat and bone meal or of cattle from the United Kingdom. The epidemiological origin of BSE is relatively unimportant for countries that have imported either meat and bone meal or live cattle from the United Kingdom. The important factor is the prevention of exposure of the cattle population through potentially infected meat and bone meal. This can only be achieved by an effective ban on the exposure of cattle to ruminant-derived protein.

As is evident from the findings in the United Kingdom and in Switzerland, this prevention of exposure cannot be solely dependent on the rendering processes in use. All means of preventing the exposure of infection of cattle have to be put in place.

**Age-specific incidences**

The age-specific incidences of BSE (Table 1) have been a very important aspect of the descriptive epidemiology of BSE throughout the epidemic. In the very early stages of the epidemic and the epidemiological investigations the age-specific incidences were used to
assess the results and fit of the simulation studies with respect to the observed and predicted age-specific patterns of disease. The age-specific incidences of BSE have also been of importance in understanding the dynamics of the epidemic as a whole. The effects of recycling infected cattle tissues are evident from these age-specific incidences. For example, the incidence in seven-year-old animals was greater in 1995 than in previous years and only showed a decrease in 1996. This can be explained by the fact that animals reaching seven years of age in 1995 would not have been affected by the feed ban, reflecting the apparent age-dependent susceptibility. The first effects of the feed ban of July 1988 became apparent from the change in incidence in two-year-old animals in 1991 (Wilesmith and Ryan, 1992) and subsequently in three-year-old animals in 1992 (Wilesmith and Ryan, 1993) before the decline in the national incidence in 1993. The reduction in incidence in these two younger age groups did not affect the national incidence because the contribution of clinical cases in the two- to three-year-old animals to the annual incidence was small. Because of the effects of recycling, the fact that most animals become infected in the first six months of life and the mean incubation period of 60 months, the incidence in the older animals increased. This more than counteracted any reduction in the incidence in the younger age groups. It was therefore not until the feed ban affected the incidence in animals four years old and greater that the epidemic declined. A study of the age-specific incidences highlights the difficulties of determining the effects of any control measures on a disease with a long incubation period when the risk of infection was increasing exponentially when the control measures were introduced.

Table 1: Age-specific incidences of confirmed cases of BSE in adult animals in affected herds with home-bred cases, by year (percentage)

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The age-specific incidences have also provided a good insight into the incubation period distribution for modelling studies (Richards et al., 1993) because most cases result from infection in calfhood. The apparent incubation period has not changed significantly throughout the epidemic. This is fortunate, but perhaps unexpected. In serial passage of scrapie in laboratory strains of mice the incubation period declined during the first few passages, but eventually attained constancy. This phenomenon has not been observed over the course of the BSE epidemic.

The mean ages at onset for 12-month (July to June) birth cohorts has provided some supporting evidence for the start of exposure in 1981182 (Table 2).

Cases in the earlier born cohorts were considerably older at the onset of clinical signs. This is consistent with these animals being infected in adulthood rather than in calfhood, as they would have been born before the start of exposure. The more recently born cohorts are subject to truncation as more cases will occur in them a the mean ages will increase.

**Herd aspects of BSE**

In the United Kingdom BSE has been predominantly a disease of dairy herds rather than beef suckler herds because it is more common to feed dairy herds commercial feedstuffs, which were likely to contain meat and bone meal. 1 October 1997, 60.2 percent of dairy herds in the United Kingdom (excluding Northern Ireland) had experienced at least one case of BSE compared with 15.9 percent of beef suckler herds. The latter percentage overestimates the true risk for animals born in beef suckler herds, as the majority (70 percent) of affected suckler herds have shown cases in purchased cross-bred animals originating from dairy herds. The difference in risk between these two basic types of herd is simply explained by the use of commercial concentrated feedstuff which is far lower in beef suckler herds.

The annual within-herd incidence has been relatively low even at the peak of the epidemic, when it was approximately 3 percent of adult animals within affected herds (Table 3). Cumulatively, 5 1.1 percent of the affected herds have only experienced one or two cases.

**TABLE 2: Mean ages at onset of clinical signs by12-month (July to June) birth cohorts**

<table>
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<th>Birth Cohort (July-June)</th>
<th>Number of cases</th>
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</tbody>
</table>
As indicated previously, the epidemic in cattle has undoubtedly been driven by the recycling (infected cattle tissues via meat and bone meal). The prevalence of infected cattle entering this part of the food chain would have increased exponentially until the feed ban in July 1991. However, the way in which the incidence of clinical cases increased in the early part of the epidemic has been of particular interest. One would expect a higher effective dose to increase both the number of affected herds and the incidence within affected herds. As indicated in Table 3, the latter has changed little, but there was a large increase in the number of affected herds coincident with the number of cases (Figure 4).

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977/78</td>
<td>10</td>
<td>147.2</td>
</tr>
<tr>
<td>1978/79</td>
<td>14</td>
<td>131.7</td>
</tr>
<tr>
<td>1979/80</td>
<td>62</td>
<td>118.0</td>
</tr>
<tr>
<td>1980/81</td>
<td>129</td>
<td>107.1</td>
</tr>
<tr>
<td>1981/82</td>
<td>387</td>
<td>93.5</td>
</tr>
<tr>
<td>1982/83</td>
<td>1986</td>
<td>81.0</td>
</tr>
<tr>
<td>1983/84</td>
<td>5265</td>
<td>72.9</td>
</tr>
<tr>
<td>1984/85</td>
<td>8279</td>
<td>68.4</td>
</tr>
<tr>
<td>1985/86</td>
<td>12521</td>
<td>67.0</td>
</tr>
<tr>
<td>1986/87</td>
<td>23110</td>
<td>65.4</td>
</tr>
<tr>
<td>1987/88</td>
<td>38902</td>
<td>62.8</td>
</tr>
<tr>
<td>1988/89</td>
<td>16175</td>
<td>63.7</td>
</tr>
<tr>
<td>1989/90</td>
<td>10582</td>
<td>61.9</td>
</tr>
<tr>
<td>1990/91</td>
<td>4333</td>
<td>59.2</td>
</tr>
<tr>
<td>1991/92</td>
<td>2739</td>
<td>54.9</td>
</tr>
<tr>
<td>1992/93</td>
<td>712</td>
<td>48.8</td>
</tr>
<tr>
<td>1993/94</td>
<td>38</td>
<td>41.0</td>
</tr>
</tbody>
</table>

*Data accumulated by 3 October 1997*
### Table 3: Annual within-herd incidence of BSE

<table>
<thead>
<tr>
<th>Year</th>
<th>Within-herd incidence (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>2.0</td>
</tr>
<tr>
<td>1989</td>
<td>2.2</td>
</tr>
<tr>
<td>1990</td>
<td>2.7</td>
</tr>
<tr>
<td>1991</td>
<td>3.1</td>
</tr>
<tr>
<td>1992</td>
<td>3.5</td>
</tr>
<tr>
<td>1993</td>
<td>3.1</td>
</tr>
<tr>
<td>1994</td>
<td>2.8</td>
</tr>
<tr>
<td>1995</td>
<td>2.4</td>
</tr>
<tr>
<td>1996</td>
<td>2.2</td>
</tr>
<tr>
<td>1997</td>
<td>1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage refers to animals that have calved at least once.

This means that the average dose of infectivity was extremely low and the main effect of recycling was to increase the number of batches of meat and bone meal with the minimum "threshold" amount of infectivity necessary to infect cattle, rather than the concentration of infectivity within batches. The details of this recycling are the subject of current modelling studies as this phenomenon is clearly important to elucidating the epidemiology of CJD. However, the initial interpretation that BSE was the result of a low dose exposure (Kimberlin and Wilesmith, 1994) has now been established by laboratory studies.
Experimental oral exposure using 1 g of brain, wet weight, from a terminal case results in disease with an incubation period within the range observed naturally.

The within-herd incidence requires careful interpretation as it cannot be used to estimate the actual attack rate. The distribution of the infectious agent in meat and bone meal would not have been homogenous, and feeding regimes and feed suppliers would have varied both within individual herds and from herd to herd, from year to year. However, a theoretical approach has provided some quantitative perspective of the attack rate. If the average dairy herd has 70 adult cows and the annual replacement rate is 20 percent, then each new birth cohort joining the adult herd would comprise 14 heifers. A single case in the cohort thus represents an attack rate of 7 percent (Wilesmith, 1991).

Another important feature of the epidemiology of BSE is that of all affected herds up to October 1997, 50 percent only had a single birth cohort affected. This is a further manifestation of the heterogenous distribution of the infectious agent in feedstuffs. The question of horizontal transmission is discussed in Chapter 5; however, this feature and the declining within-herd incidence suggest strongly that natural transmission between cattle has not occurred.

**TABLE 4**: Cumulative percentage of herds with BSE in home-bred cattle in the United Kingdom (excluding Northern Ireland), by adult herd size
<table>
<thead>
<tr>
<th>Adult herd size</th>
<th>Number of herds at risk</th>
<th>Number of affected herds</th>
<th>Cumulative incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9</td>
<td>30147</td>
<td>228</td>
<td>0.8</td>
</tr>
<tr>
<td>10-29</td>
<td>22401</td>
<td>1 120</td>
<td>5.0</td>
</tr>
<tr>
<td>30-49</td>
<td>15542</td>
<td>2993</td>
<td>19.3</td>
</tr>
<tr>
<td>50-99</td>
<td>19811</td>
<td>8162</td>
<td>41.2</td>
</tr>
<tr>
<td>≥100</td>
<td>9263</td>
<td>7641</td>
<td>82.5</td>
</tr>
</tbody>
</table>

The final herd aspect of the epidemiology of BSE is that of adult herd size. Throughout the epidemic the larger the adult herd, the greater it, risk of experiencing a clinical case. Table 4 provides the cumulative incidence of home-bred cases by herd size from the beginning of the epidemic in 1986 to October 1997. This feature is consistent with the feedborne hypothesis. The larger the herd, the more feed is required and therefore the greater the chance of buying an affected batch. It also suggests that surveillance to detect the occurrence of BSE would benefit from concentrating on large herds.

**Geographic distribution**

A remarkable feature of the start of the epidemic was the more or less simultaneous occurrence of BSE throughout England, Scotland and Wales and associated islands (Figure 5). This is consistent with a widespread common source of infection.
However, there was a marked geographic variation in incidence. In the early years of the epidemic the incidence of BSE, as determined by the cumulative percentage of dairy herds with at least one case of BSE in a home-bred animal,

The reasons for this variation in incidence were essentially elucidated from the survey of rendering plants (Wilesmith, Ryan and Atkinson, 1991). These were threefold. First, the use of solvent extraction in the production of meat and bone meal was confined to Scotland, where nearly 80 percent of production was subject to this process. Second, in the past there were a number of small rendering plants producing tallow and the fat-rich intermediate product greaves. This was sold to the large rendering plants where it was totally reprocessed, and the resulting meat and bone meal had been subject to a double heat treatment. The proportion of total production processed in this way in each geographical region was found to be negatively correlated with the regional incidences of BSE. Third, there was variation among feedstuff producers in their use of meat and bone meal and geographic variation in the market share that the producers had. This variation was relative, as all feedstuff producers had used meat and bone meal as an ingredient. This effect was and has been most evident within the Channel Islands.
Although proprietary cattle feedstuffs have been purchased from mainland England, there has been a markedly greater incidence on the island of Guernsey than on the island of Jersey. This was simply because the main producers of cattle feedstuffs imported into Guernsey used meat and bone meal more frequently than the main producer of rations imported to Jersey. This fortuitous occurrence was obviously a useful natural epidemiological experiment and assisted in establishing the meat and bone meal hypothesis. In the United Kingdom (excluding Northern Ireland) the geographic variation in incidence has changed notably since the occurrence of cases in animals born after the feed ban in July 1988. This is discussed in the following section.

**ANIMALS BORN AFTER THE FEED BAN (BABs)**

As indicated in earlier sections the incidence of BSE began to decline as expected during 1993. However, in 1991 it became evident that the feed ban was possibly not totally effective, as cases began to occur in animals born after the feed ban (BABs).

These cases have attracted much attention and speculation about the natural transmission of
BSE between cattle, either maternally or horizontally. Therefore, there has been considerable epidemiological research to determine the reasons for their occurrence. The distribution of these cases by their month and year of birth is given in Figure 7.

A cohort study was initiated in 1989 to examine for maternal risk factors. This was a long-term study and results were not possible before the end of 1996. Maternal transmission of BSE on its own could never sustain the disease in the cattle population of the United Kingdom. This is simply because the “contact rate” will never exceed 1:1, that is not every infected animal will produce a calf which reaches adulthood and in turn produces a calf (Wilesmith and Wells, 1991). However, studies to examine for maternal risk factors have been conducted as these are detectable earlier than any risks of horizontal transmission. They therefore potentially provide the means for determining whether or not BSE is transmissible other than by the feedborne route.

When a sufficient number of BAB cases had occurred in 1993 a case-control study was initiated to investigate a maternal risk factor and the possibility of horizontal transmission (Hoinville, Wilesmith and Richards, 1995). The results of this study did not detect a maternal risk factor, in that the offspring of animals that were subsequently affected with BSE were not found significantly more often among the cases. There was a marginally statistically significant risk for animals born up to three days after a subsequently affected animal calved, but this was considered to be unlikely to indicate a causal association. Most important, the result of this
case-control study indicated that neither maternal nor horizontal transmission could account for the majority of cases in animals born after the feed ban.

During and subsequent to the course of this study epidemiological analyses of the main database together with in-depth studies of BAB cases have been conducted to quantify the effects of the feed ban and to determine the reason for the occurrence of cases in such animals. The effect of the feed ban has been examined by both modelling studies and the use of standardized morbidity ratios. These rates have provided a relatively simple means of monitoring the effects of the epidemic (Hoinville, 1994). This analysis allows for the fact that cases will accrue to any birth year cohort- the problem of right truncation (Figure 8).

**FIGURE 8**

Standardized morbidity ratios indicating the risk of infection for animals born in succeeding birth cohorts compared with animals born in the 12-month (July to June) birth cohort before the feed ban in July 1988

In this analysis the risk of developing BSE for an animal born in any month and year after the feed ban in July 1988 was compared with the risk of disease for an animal born in the same month in the 12 months (June 1987 to July 1988) before the ban, allowing both cohorts the same time to develop disease. For example, in June 1996 the risk of disease occurring in animals born in June 1990 could be determined using the data accumulated for this cohort and compared with data for cases accumulated by June 1994 among animals born in June 1988. The underlying assumption in this analysis is that there has been no change in the incubation period distribution in successive birth cohorts since 1988. No such change is apparent. The results are shown in Figure 8 and indicate that there was a more or less instant, but not complete, effect for animals born in August 1988. This may have been unexpected, but cattle feedstuff producers had been forewarned of the introduction of the ban on the feeding of
ruminant-derived protein and would have therefore been able to conform with the ban in a timely manner. In subsequent birth cohorts the risk of developing disease has declined. The risk of becoming infected for animals born in December 1990 was 10 percent of the risk for animals born in December 1987. The risk did not decline significantly for animals born in 1991, 1992 and 1993. Although the feed ban was effective it was not completely so - therefore additional studies were required.

The in-depth case studies of affected animals born in the second half of 1988 indicated that they were at risk from feedstuffs in the feed chain or on farm which had been manufactured before the ban and contained ruminant protein. Such feedstuffs could not be detected. This source would have been essentially exhausted three to six months after the ban, but it was likely to be the source for the majority of affected animals born in the second half of 1988.

The reasons for the continued exposure to the feedborne source, albeit at a reduced level, started to be identified with analysis of the geographical variation in incidence. An analysis of the regional distribution of home-bred cases born in 12-month (July to June) birth cohorts revealed an interesting change (Table 5). This analysis was restricted to home-bred cases, as finished feedstuffs are distributed within relatively short distances and the objective was to locate where the continued risks from feed were greatest.

The important finding was the increase in the proportional occurrence of BSE in animals born after the feed ban in the northern and eastern regions of England. The regions contain a substantial proportion of the pig and poultry populations of the United Kingdom, the diets of which were unaffected by the 1988 feed ban.

### TABLE 5 The regional distribution of home-bred cases born in each 12-month (July to June) birth cohort (July 1985 to June 1991)

<table>
<thead>
<tr>
<th>Birth cohort</th>
<th>Distribution of cases (%)</th>
<th>Total number in cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>North</td>
<td>Mid and west</td>
</tr>
<tr>
<td>1985/86</td>
<td>8.3</td>
<td>17.2</td>
</tr>
<tr>
<td>1986/87</td>
<td>9.2</td>
<td>19.3</td>
</tr>
<tr>
<td>1987/88</td>
<td>9.9</td>
<td>19.1</td>
</tr>
<tr>
<td>1988/89</td>
<td>11.4</td>
<td>21.2</td>
</tr>
<tr>
<td>1989/90</td>
<td>12.6</td>
<td>21.2</td>
</tr>
<tr>
<td>1990/91</td>
<td>16.4</td>
<td>19.3</td>
</tr>
</tbody>
</table>
A further analysis, on a county basis, revealed statistically significant correlation between the cumulative incidence of BAB cases and the ratios of both cattle to pigs and cattle to poultry. This has resulted in a change in geographical cumulative incidences of dairy herds with only home-bred BAB cases (Figure 9).

Figure 9 can be compared with Figure 6 and highlights the enhanced risk in the northern and eastern regions of England for the occurrence of BAB cases.

Subsequent investigations and the use of an enzyme-linked immunosorbent assay (ELISA) to detect species-specific protein have revealed why there was a continued feedborne source. There is no evidence for any extensive deliberate and illegal use of ruminant protein in ruminant rations. The problem has been a result of accidental cross contamination of ingredients used for cattle feedstuffs, and of the products themselves, with ruminant protein which was present in the feed mills for use in pig and poultry rations; the majority of feed mills produced multi species, both ruminant and monogastric, rations. This cross contamination is unlikely to have involved large quantities of meat and bone meal because of the fact that the effective exposure of cattle is equivalent to less than 1 g of brain, wet weight from terminal cases of BSE (G.A.H. Wells am A.R. Austin, personal communication). Two possible points of cross-contamination in the production process were probably most important: The first was the point of delivery of feed ingredients; frequently a single portal of entry was used, and remnants of deliveries of meat and bone meal may have been left in augurs and on conveyor belts, which were then used to transport ingredients for cattle rations. The other was the use of poorly pelleted pig feedstuffs in cattle rations because of the less demanding nutritional requirements of cattle.
There were other means of exposure to infected feedstuffs after their commercial production. Examples are the spillover of pig and/or poultry rations in delivery lorries and the feeding of cattle with feedstuffs produced for pigs and poultry. In Switzerland, where there have also been minor problems with the feed ban, the access for cattle to feed stores on farms containing pig and poultry rations may have contributed to continuing this risk (B. Hönnlimann, personal communication).

Such accidental cross-contamination should not have occurred after September 1990, when SBOs, considered to contain the highest concentration of the BSE agent, were banned from the rations of all animal species (HMSO, 1990). However, it became apparent that there was incomplete compliance with this SBO ban. This was rectified in 1995, and in March 1996 any remaining risk from the feedborne source was cut off by the ban on the feeding of any mammalian protein to any farm animal species (HMSO, 1996).

The conclusions from these investigations indicate the importance of the low-dose risk phenomenon in the occurrence of BSE. More important, the lesson to be learned is that all efforts and associated legislation need to be directed at closing all channels of potential exposure of cattle to the feedborne source. The initial feed ban of July 1988 had a dramatic effect and given time would have proved to be totally effective, in the absence of other means
of transmission, because the prevalence of infected cattle entering the recycling process (i.e. via meat and bone meal) was reduced each year. Given the occurrence of \textit{nv}CJD cases and the evidence, through strain typing (Bruce \textit{et al.}, 1997), of a link with the occurrence of BSE, the feedborne source needed to be stopped immediately and totally - hence the total ban introduced in March 1996. This aspect of the epidemiology is discussed in a subsequent section concerned with the risks of BSE occurring in countries outside the United Kingdom (see Chapter 4).

**INVESTIGATION OF A GENETIC ORIGIN**

Initial epidemiological studies were concerned with determining whether or not BSE was a solely genetic disease. There was no evidence for this (Wilesmith \textit{et al.}, 1988). Subsequent studies addressed the likelihood that there was a genetic control of the susceptibility of cattle to BSE analogous or similar to that which has been gradually unraveled, with the assistance of molecular genetic techniques, for sheep and their susceptibility to scrapie.

The first indications that BSE in cattle may be different from sheep scrapie in this respect came from experimental inoculation of cattle with brain homogenates, which resulted in all cattle exposed succumbing to clinical disease with an identical incubation period (Dawson, Wells and Parker, 1990; Dawson \textit{et al.}, 1990). Comparable studies of scrapie in sheep would have resulted in an incomplete attack rate, not all animals would have succumbed, and there would have been a variable incubation period. The second piece of evidence that host genetic variation may not be of great importance was the remarkably uniform pattern of severity and distribution of vacuolar lesions, compared with scrapie, on histological examination (Wells and Wilesmith, 1989; Wilesmith and Wells, 1991). Third, although the epidemiological picture of the natural disease in the field suggested there was a heterogenous distribution of the BSE agent in the feed supply, and the annual within-herd incidence was low (see above), a significant proportion, and in some herds all members, of a birth cohort had succumbed to BSE. A hint that all cattle were equally susceptible was supported by the lack of any evidence of a breed predisposition.

Subsequently, molecular genetic and population genetic studies have failed to reveal any evidence for a major genetic component in the risk for cattle of developing BSE, and polymorphisms of the PrP gene (encoding the incriminated PrP protein as a component of the infectious agent) analogous to those identified in sheep (Hunter \textit{et al.}, 1994).

**THE ORIGIN OF BSE**

The working hypothesis for the origin of BSE is sheep scrapie (Wilesmith \textit{et al.}, 1988; Kimberlin, 1993; Kimberlin and Wilesmith, 1994; Kimberlin, 1996). This hypothesis arises from the original considerations with respect to the reason for only the cattle population in the United Kingdom experiencing a major incidence of BSE (Wilesmith and Wells, 1991). The risk factors originally identified were fourfold: a large ratio of sheep to cattle population, approximately 4:1, larger than in any other country; a sheep population endernically infected with scrapie, although the scrapie status of the sheep population in the United Kingdom may not be unique in this respect (this aspect on its own was unlikely to be important without the presence of the other
risk factors); the feeding of ruminant-derived protein to cattle; and conditions of rendering which allowed the effective exposure of the cattle population to a scrapie-like agent.

An examination of these risk factors in countries outside the United Kingdom indicated that they were not present in combination anywhere else, such that a major epidemic of BSE was unlikely. For example, in France the ratio of cattle to sheep is the reverse of that in the United Kingdom, although the prevalence of sheep scrapie infection may be quite similar. Similar risk assessments have been made for the occurrence of BSE in Argentina (Schudel et al., 1996) and the United States (Walker et al., 1991; Bleem et al., 1994). The risks of BSE occurring in other countries is discussed in a little more detail in Chapter 4.

This international comparison of the above basic risk factors together with the key features of the epidemiology of BSE in the United Kingdom provide supporting evidence for the sheep scrape origin hypothesis. However, alternative hypotheses of the origin of BSE have attracted considerable attention, particularly those related to a possible cattle origin (see Wilesmith et al., 1988; Kimberlin, 1993; Kimberlin and Whilsmith, 1994; Kimberlin, 1996), which are summarized here.

The major requirement for a cattle-origin hypothesis to be consistent with the start and subsequent development of the BSE epidemic is that a geographically widespread reservoir of infection was naturally maintained in the cattle population of the United Kingdom. This infection was essentially non-pathogenic within the commercial life span of cattle although rare sporadic disease could have occurred at an incidence below the inevitable detection threshold of the routine animal disease surveillance system present in the United Kingdom. A further requirement is that changes in rendering conditions that led to sufficient feedborne exposure of cattle to initiate the epidemic converted the avirulent infection into one that produced clinical disease in a large number of animals.

If one considers the hypothesis that BSE has occurred as a sporadic disease at low incidence comparable to that of sporadic CJD in human populations around the world, this would result in an annual rate of one case per million cattle. By definition this incidence would be geographically widespread and would result in four cases per year in the United Kingdom. However, the probability of such undetected cases being incorporated into meat and bone meal used in cattle feed would have varied between 30 and 10 percent, depending on the season of the year. It is unlikely therefore that a naturally occurring sporadic incidence of BSE in the cattle population could generate an epidemic because of the improbability of such a low prevalence of infected cattle tissues being recycled via meat and bone meal and being fed to cattle. Indeed the necessary amplification factor for cattle-to-cattle transmission by this means is present in countries such as the United States, and the absence of BSE there is important in this respect. The hypothesis of sporadic, CJD-like occurrence of BSE in cattle is therefore not consistent with the findings.

Consideration of the other major contending bovine-origin hypothesis -that of a geographically widespread, high-prevalence avirulent infection of cattle - requires an appreciation of the current, accepted knowledge of the pathogenesis of scrapie and related TSEs. The first aspect of this accumulated research is that the occurrence of infected carriers that do not develop
disease has long been suspected to play a role in natural scrapie (Kimberlin, 1993). They are an inevitable consequence of the modal and median age of onset of scrapie, three to five years, which is close to the commercial life span of most breeds of sheep, and naturally subject to the competition from other fatal diseases or those requiring premature culling. Carriers of the scrapie agent are to be expected in sheep because, besides the incubation period, the occurrence of clinical disease depends on two interacting risk factors: the strain of the agent and the genotype of the host. Most of the scrapie strains isolated in mice and hamsters are neuro-invasive and neuropathogenic, but there are major differences in the incubation period depending on the strain of the agent and the genotype of the host. An extreme case is a scrapie model in mice, the 87V strain (Bruce, 1985; Collis and Kimberlin, 1985). In this model, the intraperitoneal injection of low doses into mice can result in early infection and replication in the lympho-reticular system, but the recipient mice do not develop clinical scrapie in their normal lifespan. This is because the 87V strain of the scrapie agent cannot migrate from the lympho-reticular system to the central nervous system. Direct inoculation of the 87V strain into the central nervous system does, however, result in replication. This model is worthy of consideration in the bovine-origin hypothesis of BSE, specifically how such a stable carrier state could be broken.

There are two ways a stable carrier state could be broken. A germ-line mutation in the PrP gene could allow an avirulent strain of the scrapie agent to become neuro-invasive or neuropathogenic. This is unlikely as there is no evidence that the bovine PrP gene exhibits the polymorphisms reported in sheep, mice and humans, and none is associated with the occurrence of BSE. As indicated in the previous section, there is no significant genetic influence in the susceptibility of cattle to BSE.

A more likely explanation is a mutation of the avirulent agent to produce a virulent strain. The geographically widespread distribution of the earliest BSE cases requires that such a mutation occurs quite often in cattle.

However, the cattle-origin hypothesis is not supported by the fact that there have been no major epidemics in other countries. It would have been expected that the international trade in breeding cattle over decades would have resulted in avirulant endemic infection of cattle in other countries, but this has not occurred. Investigating the origin by means of laboratory-based studies is relatively complex, but long-term studies involving the oral exposure of cattle to sheep scrapie are in progress.

3. Animal TSEs related to BSE

Cases of spongiform encephalopathy (SE) in domestic cats, various species of Felidae in zoos and exotic ruminants have occurred concurrently with the BSE epidemic. Those occurring in the United Kingdom are summarized in Table 6.

The cases in nyala and gemsbok are of some interest as they both occurred in the
same zoological collection in the south of England (Jeffrey and Wells, 1988; Wilesmith et al., 1988). These animals were infected as a result of the use of meat and bone meal in their concentrate rations (for a very short period), because of a shortage of soya as the protein source. One notable feature of the disease in these two species and the other exotic ungulates has been the age at onset, which has been much younger than cattle with BSE. The observed clinical duration of SE was much shorter on average than for BSE, but this may be due to difficulties in the observation of these animals.

The cases of SE in the greater kudu, Arabian oryx, eland, scimitar oryx and the bison were all likely to be a result of infection from commercial feedstuffs containing meat and bone meal, and, in some cases, proprietary cattle rations. One of the causes in a greater kudu was the offspring of one of the affected kudu. This was initially considered to be the possible case of maternal transmission (Kirkwood et al., 1992). However, subsequent investigation revealed that this case was most likely to have become infected from cattle feedstuffs.

**TABLE 6: Spongiform encephalopathies in domestic cats and exotic species in the United Kingdom (excluding Northern Ireland)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number affected</th>
<th>Date of onset of clinical signs or confirmation of first case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyala (Tragelaphus angasi)</td>
<td>1</td>
<td>June 1986</td>
</tr>
<tr>
<td>Gemsbok (Oryxgazelia)</td>
<td>1</td>
<td>June 1987</td>
</tr>
<tr>
<td>Arabian oryx (Oryx leucoryx)</td>
<td>1</td>
<td>March 1989</td>
</tr>
<tr>
<td>Greater kudu (Tragelaphus strepsiceros)</td>
<td>6</td>
<td>August 1989</td>
</tr>
<tr>
<td>Domestic cat’</td>
<td>81</td>
<td>November 1989</td>
</tr>
<tr>
<td>Eland (Taurotragus o”)</td>
<td>6</td>
<td>December 1989</td>
</tr>
<tr>
<td>Puma (Felis conColo~b)</td>
<td>3</td>
<td>Early 1992</td>
</tr>
<tr>
<td>Cheetah (Acinonyxjubatus)c</td>
<td>4</td>
<td>September 1992</td>
</tr>
<tr>
<td>Scimitar oryx (Oryx damma)</td>
<td>1</td>
<td>December 1992</td>
</tr>
</tbody>
</table>
The low-incidence epidemic of spongiform encephalopathy in domestic cats (FSE) has allowed an epidemiological study, but the small number has precluded analytical approaches. A standard epidemiological questionnaire has been used for the FSE cases. Analysis of these data have revealed a widespread geographical distribution and a similar clinical duration and mean age at clinical onset as for BSE. Suspected clinical cases of FSE are not statutorily notifiable, but analyses have indicated that a major epidemic has not gone undetected. The true peak annual incidence was probably of the order of 14 cases per million cats (Wilesmith, unpublished findings). Investigations of the possible source and means of infection of cats suggest that commercially produced cat food is the most likely source. The legislation introduced in September 1990 banning the use of SBOs (considered likely to contain the highest concentration of the BSE agent) in any animal feedstuffs would have prevented further exposure (HMSO, 1990). In addition, the pet food industry had already instituted a voluntary ban on the use of high risk tissues in 1989. These bans have been effective, as the incidence began to decline during 1996 and 1997 (Wilesmith, G.A.H. Wells and J.B.M. Ryan, unpublished findings). There has only been one case of SE in a cat born after the statutory ban - infection may have resulted from the relatively long shelf-life of canned products (introduced before the ban).

The first cases of SE in the large cats in zoological collections occurred slightly later than in domestic cats. However, the average age at onset for the exotic Felidae has been older than that for the domestic cats. In the cases that have been investigated in full, the exotic Felidae became infected from being fed raw bovine tissues, including spinal cord or brain. In one zoological collection there is likely to have been a selection of BSE cases in the very early years of the epidemic, when the awareness of the occurrence, of BSE was minimal, because, as a protectionary measure, they used only fallen stock of cattle that had died of metabolic diseases (hypomagnesaemia and hypocalcaemia are important differential diagnoses for

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiger</td>
<td>Pathera tigris</td>
<td>1</td>
</tr>
<tr>
<td>Bison</td>
<td>Bison bison</td>
<td>1</td>
</tr>
</tbody>
</table>

4. BSE in countries Outside the United Kingdom

The numbers of cases of BSE in countries outside the United Kingdom, by November 1997, are given in Table 7.

In Ireland, infection was imported in cattle (Griffin et al., 1997). The cattle population in Switzerland, the other country that has experienced a very low-incidence
epidemic, was most likely infected by imported meat and bone meal. Relatively large amounts of meat and bone meal were imported from other countries, which could have arisen from the United Kingdom, but only a very small quantity was purchased directly from the United Kingdom (Hbmliman and Guidon, 1994). A small number of cases of BSE have occurred in animals born after the ruminant feed ban in Switzerland, which have been attributed to the problem of cross-contamination within feed mills.

The reason for the occurrence of relatively small numbers of cases of BSE in indigenous cattle in other countries (France, Portugal, the Netherlands, Luxembourg and Belgium) is uncertain, but it may result from imported meat and bone meal or the incorporation of tissues from infected cattle, imported from the United Kingdom, for the production of meat and bone meal.

**TABLE 7: Cases of BSE in countries outside the United Kingdom**

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of cases</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indigenous animals</td>
<td>Imported animals</td>
</tr>
<tr>
<td>Belgium</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Canada</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Falkland islands</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Ireland</td>
<td>241</td>
<td>12</td>
</tr>
<tr>
<td>Italy</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Oman</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
There has been an estimation of the risk of BSE occurring in animals exported from the United Kingdom to European countries, which were members of the European Community in 1989 (Schreuder et al., 1997). An important part of the estimation process was to determine the potential exposure of the human populations in these countries to the BSE agent, in view of the occurrence of CJD. The results indicated that there may have been an incomplete ascertainment of cases of BSE in imported animals. This is probably inevitable given the non-specificity of the initial clinical signs (see Chapter 6) and the fact that BSE occurs at a low within-herd incidence, frequently as single cases. However, it is important to realize the magnitude of the risks of the introduction of infected cattle, because of the potential inclusion of their tissues in meat and bone meal within the recipient countries. Rendering systems that are capable of reducing the concentration of the BSE agent in the resultant meat and bone meal have been identified (Taylor, Woodgate and Atkinson, 1995), However, it is important to recognize that no study can identify a rendering system (in terms of particle size, temperature and processing time) that can totally inactivate the BSE, or other TSE, agent. Only reduction in the concentration of the agent can be identified by a study. Therefore countries that could have been "seeded" with the BSE agent, by the past importation of cattle or meat and bone meal from the United Kingdom, should have all control in place measures (see Chapter 8), and not rely on

5. The future course of the epidemic in the United Kingdom

Figure 3 (p. 12) indicates that the BSE epidemic in the United Kingdom is in rapid decline, with an approximate 40 percent reduction each year. If this continues, the incidence of BSE will be very low in the early 2000s. It is impossible to predict the tail of the epidemic, and therefore the year of the last case, because of the protracted incubation period. Long incubation period cases could occur well into the future, as has been the case in the kudu epidemic in Papua New Guinea.

In refining the predictions of the future course of the BSE epidemic in the United Kingdom, much of the epidemiological research has been concentrated on determining whether or not there are other means of transmission of the BSE agent than by the feedborne source. As described previously, the occurrence of cases in animals born after the feed ban could not be attributed, in the main, to horizontal or maternal transmission from the case-control study (Hoinville, Wilesmith and Richards, 1995). The results of the cohort study to examine maternal risk factors became available in 1997 (Wilesmith et al., 1997; Curnow, Hodge and Wilesmith,
1997; Donnelly et al., 1997a; Donnelly et al., 1997b). This study was unpredictably confounded by the continuation of the feedborne source, because the cross contamination problem was not apparent at the onset of the study. Cross-contamination has caused some problems in the interpretation of this study, in disentangling the occurrence of true maternal transmission from a possible genetic factor not related to susceptibility, such as the genetic influence of feed intake.

A further epidemiological study was made of the offspring of beef suckler cows affected by BSE in the United Kingdom that were kept in close contact with their dams and consumed their milk for the first six months of their lives, which is relevant to the assessment of maternal transmission (Wilesmith and Ryan, 1997). In this study no cases of BSE have occurred in these offspring. This suggests that true maternal transmission may not occur, and the maternal effect observed in the cohort study may have been a result of exposure to the feedborne source. This is because concentrate feeds are used far less in beef suckler herds than in dairy herds. However, if the results of the cohort study are taken at face value, a maternal rate of 10 percent, this will not significantly prolong the epidemic nor affect the future incidence.

There is no evidence for horizontal transmission of BSE in herds in the United Kingdom. The within-herd incidence has been closely monitored, and has decreased coincidentally with the national incidence (Table 3, p. 15). In a number of herds the incidence has been reduced to zero following a change in feed supplies to exclude meat and bone meal in cattle feedstuffs. In addition, the relatively small number of herds that have had a large number of cases is useful in this respect.

Cumulatively, there have been 48 herds in the United Kingdom which have experienced 50 or more confirmed cases of BSE and a total of 3,023 cases. The average number of cases per herd was therefore 63, a relatively large potential weight of infection. Nothing can be concluded from the continued incidence of BSE in these herds on the question of horizontal infection. However, in 15 of these 48 herds, the last cases (as at October 1997) were born before 1 July 1991 - a period in excess of the mean and median age at onset has been exceeded. The accumulated evidence from this and the other epidemiological findings therefore do not provide any cause for concern for the future course of the epidemic.

In summary, it is likely that a continuing ban on the use of mammalian protein in animal feedstuffs will decrease the incidence of clinical disease, and eventually the disease will become extinct. Yet, sporadic cases may occur in the future as a result of the long incubation period of B,
6. Clinical signs

Although surveillance for BSE using the histological examination of the brains of animals in high-risk categories is important, an appreciation of the clinical signs of BSE is fundamental to the front line of a surveillance programme.

The initial clinical signs of BSE are non-specific and may be detected only by the animal's keeper because the signs are more behavioural than neurological in nature and are only recognizable with repeated observations and knowledge of the animal's normal behaviour frequencies of the basic initial clinical signs recorded during the BSE epidemic in the United Kingdom; it includes those recorded at a frequency greater than 1 percent.

In recording clinical histories, it is important to establish how frequently the animal
has been observed and to note the stage of the reproduction cycle of the animal. For example, cows in their non-lactating period tend not to be observed as frequently as lactating cows, and are not subject to the stimulatory factors associated with the milking parlour, which can enhance the manifestation of clinical signs. The production type of the cow, dairy or beef stickler, is therefore important in this respect. The important initial signs are: separation from the rest of the herd; a reluctance to enter the milking parlour, or simply a change in the order in which the animals enter it; and a change in gait, particularly hind limb locomotion, which may only be observable to the experienced herd owner. Although not shown in Figure 10, the initial sign in a very small proportion of cases will be recumbency. The early changes can be confused with metabolic diseases, particularly hypomagnesaemia, and test therapy may be both necessary and advisable.

The most important differential diagnostic aspects in the United Kingdom are the insidious onset and the chronic progression of the clinical signs. Clinical diagnosis is enhanced by repeated and prolonged examinations. Ultimately the predominant neurological signs of BSE are apprehension, hyperaesthesia and ataxia (Figure 11). Animals exhibiting a combination of these three signs for more than one month should be regarded as likely cases of BSE (Wilesmith et al., 1992).
Apprehension is mostly recognized as a fear of animal attendants. Affected animals are often notably the first to rise when someone enters a field or pen and will move away, isolating themselves by distance and place themselves the far side of the herd. As noted for the initial signs, lactating cows often adopt the last position in the milking order. This could result from general, apprehensiveness of the milking routine, a specific nervousness of entrances or visual perception problems of entrances. Animals may be observed to jump through doorways. Physical restraint often provokes varying degrees of violent or frenzied reaction, especially if the head restrained - as required for jugular venepuncture or detailed examination of the
Hyperaesthesia was a term used initially to describe the abnormal responses to the normal stimuli: sound, light and touch. This hyper-responsiveness may result from true hyperaesthesia, hyperreflexia or a combination of the two. Hyper responsiveness to touch is the most common. Prodding the trunk with a pencil or a similar-sized blunt instrument, produces vigorous panniculus contractions, marked tail movements shaking and tossing of the head and movement of the head and neck towards the flank for grooming by the tongue. Such responses may be evoked by normal tactile stimuli such as flies or strand straw or hay. This response to touch also occurs on similar stimulation of the head, the ears, nasal mucosa and especially the lower hind limbs. Stimulation of the fetlocks of the hind limbs with a broom, in a suitably restrained animal may provoke violent, repetitive and frenzied kicking. Hyper responsiveness to sound is more variable. It may be observed as a result of naturally occurring sounds, but may also be elicited by striking metal objects, resulting in widespread adventitious muscular movements such as fasciculation tremor, myoclonus and, on occasion, collapse.

Seizures may occur when adventitious muscular movements become established as a constant feature. The combined effects of apprehension and neurological hyper responsiveness are likely to be responsible for an exaggerated startle response.

Excessive licking of the nares, snorting and coughing are indicators of hyperaesthesia of the respiratory tract. Retraction of the muzzle, flehmen behaviour, is a common sign in the usual stimulation by genital secretions and their associated odours. Ear movement may be exaggerated and the resting posture of the ears may appear abnormal, either with the two ears in different positions or with both retracted caudally to their maximum extent.

Pruritus, a very common clinical sign in sheep scrapie, is not a feature of BSE. However, increased grooming of the trunk and legs is a frequent clinical sign of BSE. Head rubbing by animals with BSE is the most analogous sign to that of pruritus in sheep scrapie. This can be very vigorous and is commonly observed if selfdrinking type water bowls or other suitable metal objects are present in the animals’ housing environment. The use of the hind feet to scratch the ears is also common.

The final predominant category of neurological signs, ataxia, may be mild or absent in the early stages of the clinical phase. Hind leg ataxia is by far the most common sign, with a marked lateral sway as a notable feature. It is advantageous if animals are observed turning, as this can reveal knuckling of the fetlocks, stumbling or falling. Dysmetria may be seen in fore and hind limbs. Enforced trotting can reveal a high-stepping gait, occasionally with the tail head raised. Otherwise, the pelvis may be dropped and hind leg ataxia may be more evident. The resting posture is often exaggerated by a wide-based stance, mimicking a rocking horse appearance, with the head being carried low. The thoracic spine is often arched, with the lumbar-
sacral segment being held lower, giving the so-called "roached back" sign.

As indicated in Figure 10, more general, no neurological signs, such as weight loss and milk yield, may be the first signs observed (Wilesmith et al., 1992). This is clearly a problem in the clinical surveillance for BSE, as these signs may be quite marked and could be the reason, singly or in combination, for the premature culling of cows. The cause of these signs has not been clearly determined, but in terms of differential diagnosis on the basis of initial clinical signs, they are analogous to the distracting initial psychiatric clinical signs that have been recorded for cases of vCJD. Clinicians therefore need to be more aware of the spectrum of clinical signs ranging from non-neurological to distinctly neurological.

The working hypothesis for the two major non-neurological signs, which can occur in the early stages of BSE, is related to the reduced rumination rate. More detailed research of the clinical neurology has indicated a reduced heart rate such that more than 50 percent of measurements from BSE cases were less than 50 beats per minute. In addition, the administration of 35 mg of atropine sulphate increased the heart rate of animals with BSE, but had no effect on healthy cows. These observations have led to the suggestion that the bradycardia is mediated by increased vagal tone and could result from the pathology in the medullary vasomotor centres (Austin, Pawson and Meek, 1996). Similarly, cows affected by BSE show a reduced time spent ruminating, although eating time is maintained at normal levels. This reduction can be marked and rumination can cease (Austin and Simmons, 1993). Manual recording of the rumination rate is impractical for routine diagnosis. However, a jaw activity recorder has been used to record rumination over protracted periods of up to seven days, after which the data can be transferred to a personal computer for analysis. Such methods maybe useful in the clinical assessment of valuable animals.

The duration of the clinical disease can range from less than two weeks to as long as one year. However, the majority of cases will require euthanasia after two months (Wilesmith et al., 1992). Euthanasia is advisable as soon as there is certainty of the clinical diagnosis, as animals become recumbent and their welfare is in jeopardy. Euthanasia is most easily performed by the intramuscular injection of xylazine as a sedative followed by intravenous administration of an overdose of barbiturate.

7. Diagnosis

As indicated in Chapter 6, clinical signs can be sufficiently distinctive to provide a clinical diagnosis. However there are no practical tests currently available to detect subclinical infection in the live animal or during the clinical phase, but there is a considerable research effort
In the United Kingdom the routine diagnosis of BSE has been by histological examination of the brain. Descriptions of standard protocols for the histological examination have been produced by the European Commission (Scientific Veterinary Committee, 1994) and the International Office of Epizootics (OIE, 1996), and the following provides a summary.

The high frequency of occurrence of neuroparenchymal vacuolation in certain anatomic nuclei of the medulla oblongata at the level of the obex has provided a valid means of diagnosis on a single section of the medulla (Wells et al., 1989). However, observation of equivocal lesions in the medulla at this level requires examination of other brain areas to detect cases of BSE with potentially atypical or minimal lesions, and to establish pathological differential diagnoses.

The histopathological changes are neurodegenerative and closely resemble those of scrapie of sheep. The most prominent features are vacuolar and comprise a spongiform change in grey matter neuropil and single or multiple vacuoles within neuronal perikarya. The precise appearance of the spongiform change in the transmissible encephalopathies has been described, as observed by light microscopy (Masters and Richardson, 1978). In contrast to natural scrapie of sheep, the spongiform change in BSE is the predominant form of vacuolar change. Both forms of vacuolation are bilaterally distributed and usually symmetrical, with a constant distribution pattern throughout the brain (Wells and Wilesmith, 1989; Wells et al., 1994). Vacuoles within the perikarya, indistinguishable from those of BSE, have been reported in neurones of the red, occulomotor and other brainstem nuclei in the mid-brain, as an incidental finding. Histopathological diagnoses of BSE must not rely on the presence of occasional solitary vacuolated neurons.

DETECTION OF SCRAPIE-ASSOCIATED FIBRILS (SAF) BY ELECTRON MICROSCOPY

Disease-specific structures, termed scrapie associated fibrils (SAF), the fibrillar form of the modified form of PrP (PrPsc), were first reported, in brain extracts from affected mice and hamsters experimentally infected with scrapie, by the use of transmission electron microscopy (Merz et al., 1981; 1983).

A detailed description of the reagents and methods used to detect SAFs has been published by the research group at the Central Veterinary Laboratory in the United Kingdom (Stack, Keyes and
This technique has proved to be of great value to supplement the routine histopathological diagnosis of BSE during the epidemic in the United Kingdom. This is because the protease resistance of SAF makes it possible to isolate and identify them from brain that has undergone significant autolysis (Scott et al., 1992). It should be noted that SAF can only be purified from fresh or frozen brain, not fixed brain, and therefore fresh brain tissue should be collected from suspect cases of BSE where autolysis is evident or suspected. This has been part of the routine protocol in dealing with tissues from suspect cases of BSE in the United Kingdom. However, this should also be incorporated in the protocol for the collection of tissues from suspected cases of BSE where the disease has not yet been confirmed, or the disease has only occurred at a very low incidence.

**IMMUNOHISTOCHEMICAL LABELLING OF PrP**

The accumulation of abnormal PrP in the central nervous system is a diagnostic feature of the TSEs. Immunohistochemical detection of PrP has potential applications for diagnosis and, more important at present, the sequential pathogenetic targeting PrP pathology. Widespread use of this methodology has indicated the limitations of its sensitivity and specificity, and therefore its interpretation.

The problem arises from the fact that the primary amino acid sequence of normal PrP is highly conserved in different species; antibodies do not necessarily have to be raised against PrP from affected cattle. Therefore antibodies to PrP with both the normal and disease-specific isoforms of the protein and controls are required which either exclude or identify reactivity with the normal isoform.

Given these difficulties and reservations, reproducible immunohistochemical demonstration of disease-specific PrP has been obtained in routinely prepared histological material for BSE, and other TSEs such as scrapie and FSE. The role of this diagnostic technique will undoubtedly evolve with further research.

**DETECTION OF ABNORMAL PRION PROTEIN (PrPsc) BY WESTERN IMMUNOBLOTTING**

Biochemically, the main constituent of SAF and PrPI, is a protease-resistant neuronal membrane glycoprotein, which is disease specific
and is an abnormal, post-translational isoform of the host protein (PrP). The normal isoform is present in a wide range of tissues, and notably in the central nervous system, and is distinguishable from PrPsc because the latter forms disease-specific fibrils and is relatively resistant to protease digestion.

Immunoblotting is a method to detect the purified, protease-resistant, abnormal protein by its molecular weight and reaction with specific antibodies. The methods and reagents required to conduct this technique have been described in detail (Stack, Keyes and Scott, 1996).

For the diagnosis of BSE, Western immunoblotting can only be considered as a valid diagnostic method when applied to CNS tissue, where PrPsc accumulates in relatively large concentration. It is possible that further research and development may result in the valid detection of relatively small concentrates of the protease resistant protein in lymphoreticular tissues, or other tissues that are more accessible for a clinical evaluation. In the meantime, this remains a valuable research technique, and may be of ultimate use in the diagnosis of BSE and other TSEs.

**BIOASSAY IN MICE**

The bioassay of brain homogenates in standard inbred strains of mice represents an ultimate, but protracted, means of confirming BSE. The use of such bioassays was important in confirming that the cases of BSE initially detected at the start of the BSE epidemic in the United Kingdom could be classified as ISE. However, such an expensive means of diagnosis is not justified with the benefit of the experience and research on diagnostic methods, notably the histopathology, for BSE in the United Kingdom.

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**8. Control measures**

As indicated in Chapter 1, the first piece of legislation for the control of BSE was introduced in July 1988 (HMSO, 1988a; 1988b). This was the ban on feeding ruminant-derived protein to any ruminant animals, and was based on the findings of the initial epidemiological study. The definition of ruminant protein excluded milk and milk products and dicalcium bone phosphate. The original legislation enforced a ban until 31 December 1988, as there was some uncertainty at the time whether effective exposure of the cattle
population had continued or had been interrupted for a short period of time. However, the feed ban was extended indefinitely in December 1988 and in the same legislation, on an interim recommendation of the Southwood Committee Report (1989), there was a ban on the use of milk from suspect cases of BSE for any purpose other than feeding to the cow’s own calf. This was not based on any scientific evidence that milk was a risk, but was considered a prudent measure. Subsequently, the evidence that milk is highly unlikely to be a risk has been accumulated (Wilesmith and Ryan, 1997). While not based on any scientific evidence, the legislation required that a suspect case of BSE in the late stages of pregnancy must be placed in approved accommodation while calving, and for 72 hours afterwards, and that the placenta, discharges and bedding must be burned and buried and the isolation premises cleaned and disinfected after use. The original purpose of this measure was not to prevent infection of the calf, this would be impractical, but to reduce the risk of the potential horizontal transmission of infection to other cattle in the herd. This was based on an assumption that the BSE may be transmitted in a similar way to sheep scrapie, in which there appears to be some risk of infection for sheep exposed to the foetal membranes of sheep in the late stages of the incubation period. There is no evidence that the foetal membranes of BSE-affected cattle are infectious in bioassays in mice and in cattle, in the transmission of the BSE agent through milk (Spongiform Encephalopathy Advisory Committee, 1995).

In August 1988, a slaughter policy was introduced for a compulsory slaughter policy with compensation payments being made to the owners of the animals at 50 percent of the value for confirmed cases of BSE, and 100 percent for animals for which BSE was not confirmed. Both compensation values were subject to a ceiling. This meant that affected animals were excluded from the animal, as well as human, feed chain and could therefore not contribute to the recycling effect.

The next most notable piece of legislative action, in September 1990 (HMSO, 1990), was the ban on the use of SBO, considered to contain the greatest concentrations of the BSE agent based on studies of natural sheep scrapie (Hadlow, Kennedy and Race, 1982), in all animal species’ diets. This had the objective of removing all potentially high-risk infective tissues from the animal feed chain. It was introduced following the realization that pigs were potentially at risk, because of the transmission of the disease by multiple parenteral inoculation of BSE-infected brains.
(Dawson, Wells and Parker, 1990) and the occurrence of the first case of SE in a cat. Although designed to protect species other than cattle, this measure would have assisted in reducing the exposure of cattle given the problem of cross-contamination of cattle feedstuffs.

In October 1990, cattle farmers became legally bound to keep and maintain breeding records for ten years. The maintenance of animal movement records was extended from five to ten years. This was introduced to facilitate the identity of animals related to cases of BSE and the ability to trace them.

In November 1994, amendments were made to a number of pieces of legislation. The first was to include the thymus and intestines of all bovine animals in the definition of SBOs, except those from animals under two months of age. The second was to ban the use of mammalian protein in ruminant feedstuffs, the result of a European Commission Decision (94/381).

During the early 1990s the European Commission, the Ministry of Agriculture of the United Kingdom and the European rendering industry sponsored studies to examine the effects of the various rendering systems in use in Europe on the inactivation of BSE. The results of these studies became available during 1994. These indicated that two systems used in the United Kingdom were ineffective (Taylor, Woodgate and Atkinson, 1995). Owners of rendering plants in which these systems were used had to either change to another system, or add a process with an acceptable time/temperature treatment. These changes began in January 1995. However, it was recognized that because of the difficulty of proving total inactivation of the BSE agent by experimental studies this was merely a means of strengthening the effectiveness of the feed ban.

Also during 1994 and into 1995 the evidence accumulated that cattle were still being exposed from the feedborne source, albeit at much reduced risk (Hoinville, Wilesmith and Richards, 1995), and that there was incomplete compliance with the specified bovine offal ban. As a result a number of additional measures were introduced during 1995. The first was in April enforcing the staining of the SBO with a solution of Patent Blue V (E131, 1971 Colour Index No. 42051). This was permanent and detectable by mass spectrometry. Second, in July 1995, routine monitoring of feedstuffs in feed mills was introduced using an ELISA to detect species specific animal protein. The third measure taken, in August 1995, was to enhance the legislation on the specified bovine offal ban. The main enhancements were more stringent controls on record keeping, the use of dedicated lines or plants for the rendering of the SBOs, a ban
on the removal of brains and eyes so that the whole skull is disposed of as an SBO, and a prohibition on the removal of the spinal cord from the vertebral column apart from in slaughterhouses.

The final action to prevent exposure of cattle to the feedborne source was in March 1996 when the use of mammalian meat and bone meal was banned from the feed of all farm animals. This was a result of the identification of the new variant form of G1) (Will et al., 1996) and the obvious attendant concerns on the risks for human health.

This series of events indicates the difficulty of controlling the feedborne source which in part resulted from the low dose required to infect cattle effectively, the problems of cross contamination in feed mills and the difficulties in policing the various components of a relatively complex industry and its processes.

The identification of C.1D also stimulated requests from the Council of Ministers of the European Union, in March 1996, for the authorities in the United Kingdom to reduce the future incidence of BSE and foreshorten the epidemic. These were somewhat difficult requests to fulfill as there is no diagnostic test to detect sub clinically affected animals and there is no means of determining which animals have been exposed to the feedborne source. Various suggestions were made for rather drastic total herd culls, but those were inappropriate as BSE cannot be considered as a herd disease. Instead, the requests were considered in the light of the epidemiology of the disease. The main aspect was that BSE is a birth cohort problem as the majority of animals are affected in calf hood and at the time 50 percent of affected herds had only experienced cases in one birth cohort, as a result of the heterogeneous distribution of the BSE agent in feedstuffs.

The concept of selectively culling the remaining animals in the affected birth cohorts was therefore investigated. The main problem in this was assessing the future incidence in these birth cohorts and the general question of how the disease may cluster within and between herds, especially given the geographical change in risk for cattle described in Chapter 2. There was also the practical difficulty of tracing all members of the birth records as the legislation to maintain movement records for ten years, rather than five years, was only introduced in October 1990.

The outcome was a compulsory cull of members of affected birth cohorts born between 16 October 1990 and 30 June 1993, and a voluntary cull of members of affected birth cohorts born between 1 July 1989 and 15 October 1990. This is likely to involve
approximately 100 000 animals and should have been completed by the end of 1997. Farmers will receive financial compensation for the animals slaughtered. This will not have a marked effect on the future incidence, which is likely to be a 12 percent reduction in 1997 and a 20 percent reduction in 1998 (J.W. Wilesmith and LB.M. Ryan, unpublished findings). However, this selective cull will not foreshorten the epidemic.

A further measure, to enhance the protection of the public's health, was to remove all animals over 30 months from the whole food chain, the so-called over thirty months scheme (OTMS). This commenced in April 1996 and during its first 12 months of operation resulted in the culling of 350 000 adult cows in addition to the usual annual cull of cows of 750 000. Such extra culling will not persist, but this measure could result in a reduction in incidence of 8 percent in 1997/98 (J.W. Wilesmith and LB.M. Ryan, unpublished findings).

In conclusion, a very large number of measures have been introduced to control infection of cattle (for a complete list readers are referred to the Progress Reports on B SE in the United Kingdom -MAFF, 1997). The epidemic is declining much as expected, but there may be cases in animals born in 1995 and possibly in early 1996 before the final ban on the feeding of mammalian protein to all farm animal species was introduced in March 1996. It is also impossible to predict the ultimate end of the epidemic because of the protracted incubation period, but there is some certainty that the incidence will be very low at the beginning of the new millennium.