Introduction

The recognition of bovine spongiform encephalopathy (BSE) as a novel clinical entity in 1986 started a dramatic chain of events in the United Kingdom (UK) and subsequently in other countries.

As an apparently new clinical condition, the exact nature and origin of BSE were the subject of exhaustive scientific investigations. The possible link with scrapie in sheep led over the likelihood of transmission to other species, including humans. A series of measures were introduced to control the disease with a view to its eventual eradication. These measures were progressively enhanced as knowledge of BSE and its transmission developed.

An important part of the UK response to the appearance of this disease was the establishment of a series of independent scientific committees to advise on the disease and control measures. These were the Southwood Committee, the Tyrell Committee on Research into Spongiform Encephalopathies, and the Spongiform Encephalopathy Advisory Committee (SEAC) which continues to provide independent advice.

This paper reviews the emergence of the disease and the work carried out to identify the means of transmission. The authors examine the likely origins of infection and the experimental work on aspects of disease transmission. In particular, this paper considers the mechanisms of transmission, the possibility of maternal transmission, the risk of transmission by embryo transfer and the pathogenesis of the disease in cattle. Finally, it reviews the management of the epidemic in the UK and its likely future progress.

Background

Bovine spongiform encephalopathy was first recognised in the UK as a result of routine animal disease surveillance measures. The disease was first defined as a clinical entity in November
1986 (98) but initial epidemiological investigations and the examination of archived bovine brains indicated that the first cases of the disease occurred around April 1985. The disease was identified as a new entity by its unusual clinical presentation, as a result of multiple cases in one of the earliest herds affected, and by talk among herd owners about their ‘unusual’ animals which prompted these owners to seek veterinary attention. Early recognition of the disease was aided by the close relationship between veterinary practitioners and the network of diagnostic laboratories of the UK Veterinary Investigation Service, now part of the Veterinary Laboratories Agency.

An epidemiological study to investigate potential aetiologies was conducted from June to December 1987. At the time, BSE was not compulsorily notifiable and the study relied on voluntary notification of cases by cattle veterinarians who had been alerted to the clinical signs of the disease.

Although the pathological features of the disease were similar to those of scrapie, other potential aetiologies were examined. The following potential vehicles for scrapie-like agents were considered:

- vaccines, hormones and other biological products
- direct or indirect contact with sheep and other animals
- imported cattle semen
- feedstuffs containing products of animal origin.

The other main potential aetiology was a toxic phenomenon resulting from the use of agricultural chemicals, such as herbicides and pesticides, and other pharmaceutical products, including organophosphorus preparations, synthetic pyrethroids and anthelmintics. A solely genetic origin was also investigated.

The study included nearly 200 affected herds and cases of BSE and the results eliminated all of the other aetiologies and possible vehicles except feedstuffs (106). Of the feedstuffs, two possible vehicles of infection were evident: meat-and-bone meal (MBM) and tallow. The suggestion that MBM was the primary vehicle was based initially on a consideration of the physico-chemical properties of the scrapie agent, which make it more likely to partition with the protein fraction rather than the lipids of tallow. This was further supported by the geographical variation in incidence which fitted more closely with the geographical distribution and handling of MBM as opposed to that of tallow.

The feedborne hypothesis was supported by a number of features of the epidemic. In the UK, BSE has been predominantly a disease of dairy herds rather than beef suckler herds. By October 2002, 61.7% of dairy herds in the UK (excluding Northern Ireland) had experienced at least one case of BSE, compared with 17.1% of beef suckler herds. The latter percentage is an overestimate for beef breeds since most affected suckler herds have had cases in purchased cross-bred animals which originated from dairy herds. The difference in risk between these two types of herd is explained by the greater use of commercial concentrated feedstuffs in dairy herds. In addition, the risk of 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 thus the greater the chances of buying an infected batch of feed. A case-control study of calf-feeding practices was initiated in early 1988 to investigate this hypothesis more formally. The results provided supporting evidence that MBM was indeed the source of the infectious agent of BSE and that calfhood infection was an important epidemiological component (109).

**Origin of the infection**

The original hypothesis of Wilesmith et al. was that BSE in cattle came from scrapie, a TSE which had long existed in sheep (107). Wilesmith based his theory on the following:

- data collected on the efficacy of rendering practices in the early 1980s in removing transmissible spongiform encephalopathies (TSEs)
- an increase in the size of the sheep population and hence the amount of material being processed, and
- a virtual cessation in the practice of removing sheep brains from dead stock before rendering.

Since Wilesmith developed this hypothesis, there have been three reviews of the origin of BSE in which this and other theories have been critically examined.

The first of these was the BSE Inquiry, chaired by Lord Phillips and published in October 2000 (68). The second was conducted by a committee of scientists chaired by Professor Gabriel Horn. Their report was published in July 2001 (48). The third was an opinion adopted by the Scientific Steering Committee (SSC) of the European Commission (EC) on 30 November 2001.

In assessing the various hypotheses on the causative agent, all three reports agree that the prion protein (PrP) theory, put forward initially by Griffith in 1967 (42), but built on and expanded by Prusiner in 1982 (71), remains central to BSE and other TSEs. These reports also agree that, whilst it remains important to keep an open mind, the scientific evidence for other suggested causative agents is not compelling at present. Those agents being considered include the following:

- alkaloidal glycosidase inhibitors (20)
- autoimmune disease caused by the production of antibodies to acinetobacter (39, 30)
- bacterial toxins (87)
– bacteria (7, 8, 9)
– chemical toxins (64)
– organophosphates (73, 74, 75).
– single-stranded deoxyribonucleic acid (DNA) (62)
– virino (21).

None of these theories is consistent with the epidemiological evidence. However, as noted in the three reviews, it is possible that one of these factors, or indeed other factors, may affect the susceptibility of cattle to BSE or may be implicated in the conversion of the normal cellular form of the PrP to its abnormal form. Nevertheless, although it has been shown that the conformation of cellular prion protein (PrPc) can be changed in vitro by both copper (88) and manganese (12), none of these changes has so far resulted in the infectious form of PrP associated with TSE diseases.

Although the weight of scientific evidence points to a central role for prions, there is considerably more doubt about the origin of the prions that produced BSE. Since Wilesmith offered his original ideas, he and others have contributed a number of theories to the debate. In summary these are as follows:

– bovine spongiform encephalopathy was caused by a scrapie-like agent from sheep that remains unchanged in its phenotypic expression in cattle (13, 107)
– bovine spongiform encephalopathy was caused by a scrapie-like agent that has changed within cattle during recycling of the agent (107)
– bovine spongiform encephalopathy was caused by a spontaneous genetic mutation within the PrP gene of cattle (69)
– bovine spongiform encephalopathy originated from another mammalian species infected with a TSE, possibly an African ungulate, a cetacean or a felidae, whose carcass became rendered and incorporated into cattle feed (48).

The BSE Inquiry report favoured the third of these, concluding that BSE probably originated from a novel source early in the 1970s, possibly from a cow or some other animal which developed disease as the consequence of a gene mutation. In reaching this conclusion, Phillips et al. were influenced by a number of factors, including different interpretations of the epidemiological data from the explanation offered by Wilesmith. Wilesmith concluded that the observed distribution of BSE cases was characteristic of an extended common-source epidemic, i.e. multiple outbreaks caused because the scrapie agent was not removed by rendering. Other epidemiologists believed that the data were also consistent with a single-point source, i.e. a novel agent that infected cattle and was gradually spread through recycling over a longer period than had originally been thought possible. This question is the subject of continuing epidemiological research. The conclusions of the BSE Inquiry also cited evidence of the differences between BSE and scrapie in their transmission properties, pathogenesis and host range.

The Horn report was less dismissive of the theory that BSE originated from scrapie in sheep. The report agreed with the BSE Inquiry that the BSE agent does not resemble any of the agents isolated from individual sheep with scrapie that have yet been identified (15). However, Horn et al. noted that the number of sheep tested is small and the number of strains of natural scrapie that may be present in the sheep population is unknown (48). The authors concluded that apparent differences between BSE and scrapie in transmission properties, host range and pathogenesis do not rule out scrapie as the origin of BSE.

The lack of knowledge about variations in the strains of TSE and how these strains are expressed in host species has been reinforced in a recent publication by Asante et al. (3). To date, BSE, and indeed variant Creutzfeldt-Jakob disease (CJD) and other isolates that have resulted from natural or experimental transmission of BSE in other species, have been found to exhibit only one set of molecular characteristics when examined by Western blotting (17). However, in this recent publication, the authors found that, in addition to the expected variant CJD (vCJD) pattern, BSE prions also produced a phenotype indistinguishable from that of sporadic CJD when injected into transgenic mice carrying part of the human PrP gene. This genotype encoded for the amino acid methionine at codon 129 of the gene. In other words, the mice expressed human PrP that contained methionine at codon 129, which is the same as seen in all cases of vCJD so far.

These three reviews may differ in the weight attached to individual pieces of scientific evidence and in the interpretation of some of the data. However, all agree that the origin of BSE remains unknown and may never be known with certainty.

Risk of transmission of transmissible spongiform encephalopathies

Since the association of BSE with vCJD (112), scientific attention has intensified on the potential for transmission of TSEs within and between species. When considering the implications for animal and public health, the key areas for risk assessment of BSE are as follows:

a) the risk of transmission of BSE from cattle to humans
b) the risk of transmission of BSE between cattle and how this risk may be reduced or eliminated
c) the risk of BSE being maintained in a sheep population by transmission from sheep to sheep.
To enable the quantitative assessment of these and other risks (e.g. secondary transmission between humans), it is necessary to quantify a range of factors which will influence the risk calculation. The following key factors should be considered:

– the identification of risk materials and the determination of infectivity levels in these materials
– the size of the species barrier
– the infectious dose
– the route of infection/exposure
– the strain of the agent
– the genotypes of the exposed populations

There have been variable degrees of advancement in the scientific understanding of these issues and more work is in progress.

Risk materials

The infectivity of different tissues from developing and clinical cases of BSE is described below in the section on ‘BSE pathogenesis’.

Species barrier

Naturally occurring TSEs are usually associated with individual species, e.g. transmissible mink encephalopathy in mink (Mustela vison) or sporadic CJD in humans, or with closely related species, e.g. scrapie in sheep, goats and mouflon (Ovis musinum), and chronic wasting disease (CWD) in elk (Cervus elaphus), mule deer (Odocoileus hemionus) and white-tailed deer (Odocoileus virginianus).

Natural exposure to BSE has led to disease in the following animals:

– cattle
– captive wild ungulates (55), i.e. greater kudu (Tragelaphus strepsiceros), nyala (Tragelaphus angasi), eland (Taurotragus oryx), gemsbok (Oryx gazella) and Arabian oryx (Oryx leucoryx)
– captive wild cats (55), i.e. cheetah (Acinonyx jubatus), puma (Felis concolor), lion (Panthera leo), ocelot (Felis pardalis) and tiger (Panthera tigris)
– domestic cats (118).

It is also possible to transmit the infection experimentally to other species such as mice (Mus musculus), pigs, sheep, macaque (Macaca spp.) (59), marmoset (Callithrix jacchus) (4, 5) and lemurs (Eulemur spp.) (11).

However, the results of attempts at inter-species transmission are variable and cannot be predicted, either from the phylogenetic classification of a species or from the PrP gene sequence of the recipient (113). Genetic studies to date have not identified shared characteristics that can be used to predict the sensitivity or resistance of one species to a TSE originating from another species. Studies in vitro suggest there may be an association between the closeness of the amino acid sequence of PrP in the donor and recipient hosts, demonstrated by the ability of scrapie-associated prion protein (PrPSc) to convert PrP into aggregated PrP (10, 16). However, the efficiency of heterologous species conversion is much lower than that of homologous species conversions (77).

Before the association of BSE in cattle with the occurrence of vCJD in humans, no causal relationship had been identified between a human TSE and an animal TSE. The link between BSE and vCJD is based on the characteristics of the disease in humans, particularly:

– age at clinical onset which indicates a likely period of exposure
– similarities in the appearance of PrPSc patterns demonstrated on Western immunoblotting
– similarities in the lesion profiles induced in mice
– similarities between vCJD and BSE when transmitted to macaques
– circumstantial epidemiological evidence from the geographical and temporal occurrence of the two diseases.

Studies on the PrP gene sequence (56) identified two pairs of derived substitutions shared by cattle and humans which suggested a predisposing link.

Information from laboratory studies has led to attempts to quantify the species barrier to derive factors which might be applied to risk analysis. The species barrier may be absolute; may be partial, i.e. will only affect a proportion of animals on first passage; or may result in an extended incubation period on first passage (97). In species that are not refractory these variations are largely attributed to the following:

– the origin of the TSE agent
– the PrP genotype of the recipient
– the route of infection
– the dose of infective tissue and
– (possibly) age at exposure.

Comparing the responses of cattle and mice to intracerebral (i.c.) inoculation with BSE-infected cattle brain indicates that mice are 1,000 times less sensitive to infection than cattle (32). Completion of the study has, however, suggested that the difference may be only 500-fold (G.A.H. Wells, personal communication). Further studies are in progress in the UK in which both cattle and sheep are exposed orally to decreasing doses of BSE. This work may provide some indication of the relative sensitivity of these two species if the lower threshold is reached.
Most of the information on the infectivity of tissues from BSE-infected cattle has been determined by bioassays in mice (102). However, estimates of the size of barrier between cattle and mice indicated a need to review the earlier results. Consequently, some work has been repeated by bioassays in cattle, thus eliminating the species barrier (see the section below on BSE pathogenesis). For the same reason, bioassays in lambs are in progress using a range of tissues from sheep experimentally infected with BSE.

For experimental (and bioassay) purposes, it is possible to produce mice with modified PrP genes. These mice may have human, bovine or ovine PrP gene sequences inserted into their genome. This may provide a means of overcoming the species barrier to provide a cheaper and quicker way of measuring infectivity. One recent development (79) reports mice with multiple copies of the bovine gene that are approximately 10 times more sensitive to infection with BSE than cattle and 10,000 times more sensitive than non-modified mice. Work with transgenic mice with human PrP genes has also been used to support the case for BSE as the origin of vCJD (81).

The EC SSC for TSEs currently recommends that, until more scientific data are available for risk assessments of human exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered as the worst case scenario, exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered as the worst case scenario, exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered as the worst case scenario, exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered as the worst case scenario, exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered as the worst case scenario, exposure to potentially BSE-contaminated products, a species barrier of 1 should be considered (32).

Infectious dose
Knowledge of the minimum dose of BSE agent required to produce infection in an individual animal would greatly assist risk analyses for the modelling of risks to animals and consumers, past, present and future.

The assessment of risk for disease control depends upon the most accurate knowledge available for:
– the minimum infectious dose of BSE required to produce infection in individuals of the species concerned
– the quantitative relationship between the amount of PrP\textsuperscript{Sc} detected and infectivity levels.

Calculations of infectious doses (ID) in BSE tissues have been based primarily on titrations conducted in mice by parenteral inoculation. The end result is expressed as ID\textsubscript{50}, i.e. the infectious dose that will produce clinical disease in 50% of challenged animals. For comparative purposes, and to enable uniformity of risk assessment, it is desirable to work to a standard procedure for the expression of infectivity. The EC SSC currently recommends a version of the following formula (32):

\[ \text{ID}_{50} \text{ per unit of mass} \]

For example, Mouse C57BL i.c. ID\textsubscript{50} per g.

Experimental oral exposure of cattle to a pool of BSE-infected central nervous system (CNS) has shown that cattle can be infected with 1 g of infected material (32). The brain pool is estimated to have contained approximately 10\textsuperscript{5} cattle oral ID\textsubscript{50}/g. Since no dose below 1 g was included, there was no endpoint at which infection did not occur. The work is continuing with cattle challenged orally with 0.1 g, 0.01 g and 0.001 g of infective brain pool, and transmission with an infectious dose of 0.1 g by mouth has recently been reported (S.A.C. Hawkins, personal communication).

The time required for determining infectivity by bioassay in an animal species is a constraint to acquiring knowledge of infectious doses. On the other hand, the detection of PrP\textsuperscript{Sc} is increasingly becoming the basis for most diagnostic tests for TSEs. At the present time, there is no established quantitative correlation between PrP\textsuperscript{Sc} and infectivity. If this correlation can be estimated with some accuracy this would facilitate the risk analysis process.

Route of infection
In susceptible animals the route of exposure to the TSE agent has been shown to affect the risk of infection occurring. In mouse scrapie models (54), the comparative sensitivity of different routes of exposure has been estimated, based on the number of mouse i.c. ID\textsubscript{50} doses required to produce infection, as follows:

- intravenous route 10
- intraperitoneal route 100
- subcutaneous and intramuscular route 10,000
- oral/intragastric route 100,000.

The estimates for oral exposure in mice were later reviewed (53) and the oral route was suggested to be 60,000 times less efficient than the intracerebral route. The author suggested a conservative value of 10,000 to be applied to the oral route for risk assessment. Challenge of cattle by intracerebral and oral routes using BSE-infected brain pool also led to estimates of a 100,000-fold difference in the efficiency of infection by the oral route, compared to that of intracerebral challenge (32).

Incubation periods for TSEs are much more variable for oral exposure with a fixed dose than for intracerebral inoculation (27). It is also likely that the majority of cattle in the UK epidemic were exposed to low dose levels. There is an absence of knowledge in the low dose range (32), but low dose exposure is likely to have a greater effect on the risk of infection than on the mean incubation period.

Strain of agent
Lesion profile studies have indicated a uniform BSE pathology in the brains of both experimentally and naturally infected cattle, examined at different stages of the BSE epidemic in the
UK (84, 100). The stereotypical lesion profile in cattle brain suggests that host and agent factors, including the strain of agent, are constant in the disease (100). Further evidence, based, for example, on the electrophoretic mobility of the protease-resistant core of PrP$^{\text{Sc}}$ (6, 17, 57, 58), as well as on transmission and pathology profiles in mice (14), is currently consistent with the existence of one strain of the agent.

Recent evidence, which requires further experimental evaluation, suggests that transgenic mice with a human PrP gene expressed a uniform disease profile when exposed to BSE or vCJD or sporadic CJD agents (3).

**Genotype of recipient**

The genotype of animals exposed to TSEs is known to have a major influence on the possibility of infection occurring (72, 93) and may significantly influence the length of the incubation period. The likelihood of infection occurring may also be influenced by the genotype of the host from which the infection was derived.

To assess the role played by the genotype of cattle in the BSE epidemic there have been studies of the nucleotide sequences of the bovine PrP gene and investigation of other parts of the bovine genome to establish if there are any variables linked to susceptibility or resistance to BSE (45).

Little variability in the bovine PrP gene has been reported (40). Different forms of the bovine PrP gene have five or six copies of a short guanine-cytosine rich element within the protein coding exon and the PrP gene of cattle does not appear to exert a detectable influence on the occurrence or incubation period of BSE. Similarly there are no reports that the PrP gene sequence of cattle that develop the infection exerts an influence on transmissibility of the infection to other cattle (32). There is some evidence from epidemiological analyses that there may be sub-sets of cattle with greater susceptibility to BSE (J.W. Wilesmith, personal communication). The statistical power of molecular genetic studies on these groups has been questioned (27).

**Maternal transmission**

The risk of TSEs being transmitted from mother to offspring was a subject of discussion from the beginning of the BSE epidemic in the UK. There is no convincing evidence that maternal transmission plays a part in the transmission of most of the TSEs. This is especially true for CJD, kuru and Gerstmann-Sträussler-Scheinker syndrome (78). Although both horizontal (80% to 70%) and maternal (20% to 30%) transmission are considered to occur in sheep scrapie (46), the evidence for maternal transmission of BSE in cattle is less clear.

The collection of detailed epidemiological data and information on all cases of BSE comprising the BSE Surveillance Database was initiated in 1987 (106). This included obtaining information on the identity and survival of the offspring of all cows in which BSE was confirmed, which enabled the study of maternal transmission. This led to Wilesmith et al. at the Veterinary Laboratory Agency initiating a cohort study in 1989 (111). The objective of the work was to determine the incidence of BSE in offspring from BSE-affected dams, compared to the incidence in offspring, born in the same calving season and herd, from cattle that remained free of the disease at six years of age. For the required statistical power and to accommodate for intercurrent mortality, 300 pairs of offspring were required. In the event, 301 pairs were purchased, each member of the pair having been born in the same calving season. The offspring were maintained until seven years of age unless the animal was euthanased due to intercurrent disease. The work concluded that there was a statistically significant risk difference between the two cohorts of 9.7% (range 5.1-14.2; confidence limits (c.l.): 95%) and a relative risk of 3.2 (1.8-5.9; c.l. 95%). This indicated that there was a maternally associated risk factor for the occurrence of BSE in this population of animals.

The results were further analysed by Donnelly et al. (26), Gore et al. (41) and Curnow et al. (18). These analyses suggested that the most likely explanation for the observed pattern was a combination of maternal-associated transmission of the aetiological agent plus a genetic predisposition. More importantly, the cohort study data suggested that the risk of developing BSE in the offspring increases for calves born closer to the onset of disease in the dam.

Donnelly et al. (25) also analysed the data from the Great Britain (GB) BSE surveillance database. This work further emphasised the increased risk to the offspring of developing BSE when the calf was born near to the onset of clinical signs in the dam. The work concluded that this argues for a significant component of maternal-associated transmission of the aetiological agent of BSE and offers little support for the hypothesis of genetic predisposition.

Other estimates and comparisons (M.S. Richards & J.W. Wilesmith, unpublished observations) of the observed and expected number of cases in offspring using information on dam-calf pairs have been performed. These results indicated a 10% maternally associated risk in the data accumulated by 1997.

Wilesmith and Ryan (110) further studied the data in the GB BSE surveillance database to look at the incidence of BSE in cows born to BSE-infected dams which had been suckled for at least one month. This search failed to find any cases in the 219 animals investigated that had been suckled for at least one month. The authors concluded that this would appear to indicate that the true risk to the offspring of BSE-affected dams may be less than that indicated by the cohort study.
In 1998, Donnelly (24) independently analysed the data from the suckler cow study, along with the data from the previous work. The modelling work appears to indicate that the data are consistent with a maternal transmission rate of 17.3% and less for up to eleven months before the onset of clinical signs in the dam. Furthermore, the work showed that there was a maternal transmission rate of 8.1% for up to 23 months before clinical onset in the dam. Donnelly concluded that the data are consistent with maternal transmission rates over the last six months of the maternal incubation period which may be higher than those indicated by the original cohort study and the analysis of dam-calf pairs in the main surveillance database.

However, recent modelling work appears to indicate that the risk of maternal transmission has occurred during the GB BSE epidemic in cattle. The data indicated that the risk increases when the dam is close to clinical disease and a genetic predisposing factor in affected offspring is suggested. From the cohort study, there was some evidence for a declining maternal risk with successive birth cohorts (111). Recent modelling studies indicate that the cumulative maternal risk, based on the whole GB epidemic data, is now reduced to 2%, but with a confidence interval including zero (J.W. Wilesmith, personal communication). One possible explanation is that the maternal effect may only contribute significantly to the numbers in the epidemic when the risk of infection from feed-borne exposure is high. The influence of a possible low-level genetic predisposition in offspring is not fully determined and the maternal risk factor has decreased.

These studies of the available data indicate that maternal-associated transmission may have occurred during the GB BSE epidemic in cattle. The data indicated that the risk increases when the dam is close to clinical disease and a genetic predisposing factor in affected offspring is suggested. From the cohort study, there was some evidence for a declining maternal risk with successive birth cohorts (111). Recent modelling studies indicate that the cumulative maternal risk, based on the whole GB epidemic data, is now reduced to 2%, but with a confidence interval including zero (J.W. Wilesmith, personal communication). One possible explanation is that the maternal effect may only contribute significantly to the numbers in the epidemic when the risk of infection from feed-borne exposure is high. The influence of a possible low-level genetic predisposition in offspring is not fully determined and the genetic basis for such a variation has not been identified.

Embryo transfer

Reproductive technologies have been used in animal husbandry for many years, whether in the simplest form of selective breeding or more complex forms, such as artificial insemination, in vitro fertilisation or embryo transfer. It was recognised that the interventions required for collecting and transferring embryos carried a risk of transferring conventional infections, though such a risk was determined to be minimal when appropriate disease-prevention protocols were followed (70, 89, 116). Accepted codes of practice, for example those issued by the Office International des Epizooties (OIE) (63) or the International Embryo Transfer Society (IETS) (49), now form the basis for international trade. The principles of these codes have been incorporated into EC directives for both trade and importation of embryos in the European Community. Indeed, embryo transfer is considered to represent a much smaller risk of transmitting conventional pathogens than movements of live animals.

Whilst the risk of transmitting conventional infections via embryo transfer can be minimised, the characteristics of TSEs are such that new information is required to assess the risk. The causative agents of the TSEs are not well characterised and are extremely resistant to inactivation by standard methods. Further, host genetics can strongly influence susceptibility to disease (notably in sheep and goats). Thus, personnel, procedure and instrumentation, as well as factors associated with the gametes and/or the embryo itself, potentially pose a risk for disease transmission (114, 115).

Upon the emergence of BSE, these uncertainties led to the European ban on export of bovine embryos from BSE-affectcd countries. An embryo transfer study was initiated in 1990 to determine whether embryos recovered from BSE-infected cows, including those fertilised by semen from BSE-infected bulls, would infect recipient dams and their embryo transfer offspring (117).

In the study, semen from 13 bulls, 8 with clinical BSE, was used for artificial insemination (AI) of 167 clinically affected cows in the terminal stages of BSE. The BSE status of the bull was not found to affect the proportion of viable embryos recovered. Resultant embryos were harvested seven days after AI according to IETS protocol and used for embryo transfer to 347 recipient heifers sourced from New Zealand. A total of 266 live offspring were born, of which 54.1% had a BSE-positive sire as well as a BSE-positive dam.

Neither recipients nor offspring showed signs of BSE within seven years and analysis of their brains (by histopathology, PrP immunohistochemistry and detection of scrapie-associated fibrils [SAF]) was also negative. Recipient animals which died, as well as aborted or stillborn offspring, were similarly tested and found to be negative. Non-viable embryos (i.e. those which could not be used for implantation) were tested for infectivity by i.c. inoculation in mice. None of the mice showed signs of TSE infection. Similarly, samples of uterine flushings were inoculated into mice but also returned negative results.

These findings indicated that embryos are unlikely to carry BSE and do not transmit the disease to recipients and their embryo-transfer offspring, even when these embryos are collected from donor cows at the end stages of clinical disease, when the risk of maternal transmission is believed to be highest. Nevertheless, the size of the study, although large, could not prove, like all such studies, that the risk was zero. The results therefore have to be accepted as indicating a risk close to zero, but specifically 0.9% (95% confidence interval – 0 to 1.35%) (117).
It should be noted that in this project the disease prevention codes recommended by the OIE and IETS were followed as closely as possible and the protein constituents used in embryo collection, processing and transfer medium were sourced from New Zealand. Precautions were also taken to avoid the carriage of BSE infectivity by personnel between farms. The EC SSC subsequently concluded that measures prescribed by the IETS for the transfer of bovine embryos are appropriate in protecting against transmission of BSE between cattle (36).

Bovine spongiform encephalopathy pathogenesis

The first report on the pathology of BSE in UK cattle described the appearance of scrapie-like fibrils and vacuolation in brain stem grey matter in a number of naturally affected Holstein/Friesian animals (98). These previously healthy cattle became apprehensive, hyperaesthetic and developed a mild unco-ordination in gait, which gradually became more pronounced with hypermetria and falling (98). From this first study, it was apparent that further research into the pathogenesis of this disease in cattle would be necessary.

Understanding the pathogenesis of BSE in cattle has been important for several reasons. It has been particularly relevant in defining the specified risk material (SRM) of cattle and the removal of this material from the human and animal food chains. At the beginning of the BSE epidemic, legislation was based on accumulated scientific data on the pathology of other TSE diseases of other animal food species (e.g. sheep scrapie) or laboratory rodent models. The subsequent development of scientific evidence on the pathogenesis of BSE in cattle has enabled legislation to be reviewed and refined in relation to SRM of cattle origin. Contrary to scientific evidence, the rules have usually only been extended rather than relaxed where no infectivity has been detected in tissues currently classified as SRM. European Union Commission Decision 2001/2 stipulates current day legislation for the removal of animal, including cattle, SRM from the food chain (33).

As well as identifying tissues of specified risk, knowledge of the pathogenesis of BSE in cattle has expedited the development of protocols for the diagnosis of this disease. Identification of the most affected tissues, body fluids or cell lineages throughout the course of the disease may, for example, improve the reliability of diagnostic tests for disease confirmation and also aid the development of pre-clinical disease tests, although the latter remains to be fully realised.

The pathogenesis of BSE in cattle also impinges on the risk of horizontal and vertical transmission of the disease. An understanding of the pathogenesis of BSE will enable appropriate control and cattle husbandry practices to be adopted, thus preventing further transmission of the disease. Information on this has been essential to corroborate epidemiological evidence which currently indicates that vertical and horizontal transmission are not significant pathways prolonging the BSE epidemic in the UK (111).

The results from a large-scale study into the pathogenesis of BSE in cattle have been published elsewhere (99, 101, 102, 103). A full summary of the results of this study and also subsequent studies, including those which are continuing, has recently become available to the public through the EC (35).

It is important to note that these studies have described the pathogenesis of BSE in cattle in terms of infectivity distribution, using mouse or cattle bioassay. Many studies of the pathogenesis of TSE diseases of other animal species, for example sheep scrapie, have used the presence of protease-resistant PrPc as a marker for the presence of potentially infective tissue (e.g. 51). It is interesting to note that scrapie infectivity in sheep tissues has been correlated with the presence of PrPc (76), although the correlation cannot be extended to quantify infectivity levels. Detection of PrPc by immunological methods has the advantage of speed when compared with mouse bioassay for infectivity. However, at present there are few specific studies which have related the presence of PrPc with infectivity in BSE-affected cattle (91).

The initial cattle challenge study involved the oral challenge of thirty Holstein/Friesian cattle of four months of age with 100 g inoculum each of pooled BSE brain stem, sourced from naturally clinically affected cattle. Tissues and body fluids were collected from challenged and control cattle at specified time points up to forty months post challenge. These specimens were assayed for infectivity by intracerebral and intraperitoneal injection into panels of inbred wild-type RIII or C57BL mice. All inocula tested for infectivity were prepared as 1/10 dilutions of the original starting material. Tissues sampled at specific time points and found to be infective by mouse bioassay have been reported elsewhere (35, 99, 101, 102, 103) and are summarised in Table I.

During the initial cattle challenge experiment, concerns were raised about the effectiveness of mouse bioassay to detect BSE infectivity of bovine materials, in terms of a species barrier effect. This factor prompted the reassessment of infectivity of tissues from the original pathogenesis experiment by conducting i.c. challenge of cattle, thus excluding any species barrier effect. These experiments are continuing, and interim data have confirmed infectivity only in certain tissues, including those found to be positive by mouse bioassay (35). Recent results, however, reported to the UK Spongiform Encephalopathy Advisory Committee, have demonstrated low levels of infectivity in pooled bovine tonsil collected from cattle ten months after experimental oral exposure to BSE infection. So far, only one of the recipient group of five cattle inoculated i.c. with tonsil tissue has developed the disease.
Table I
Tissue distribution of bovine spongiform encephalopathy (BSE) infectivity in cattle orally challenged with 100 g BSE brain material

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time period over which infectivity was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain: frontal cortex, caudal medulla</td>
<td>32-40 months</td>
</tr>
<tr>
<td>Spinal cord: C2-C3, T10-T11, L3-L4</td>
<td>32-40 months</td>
</tr>
<tr>
<td>Dorsal root ganglia: C3-C6, T5-T8</td>
<td>32-40 months</td>
</tr>
<tr>
<td>Trigeminal ganglia</td>
<td>32-40 months</td>
</tr>
<tr>
<td>Distal ileum</td>
<td>6-14 months, 18 months, 36-40 months</td>
</tr>
<tr>
<td>Bone marrow (sternum)</td>
<td>38 months</td>
</tr>
</tbody>
</table>

Based on current data from experimental oral challenge of cattle, the pathogenesis of BSE would appear to have a much more restricted tissue distribution in comparison to the TSE diseases of other animal food species, including sheep (1, 2, 43, 44, 51, 66, 67, 76, 95, 96), goats (65, 66) and deer (61, 82, 83).

One striking feature of the pathogenesis of BSE is the apparent reduced involvement of the lymphoreticular system (LRS) in comparison to that seen in other TSE diseases of other animals, including sheep scrapie (50, 51, 76, 94, 95, 96). The limited involvement of the LRS in cattle is not an intrinsic feature of BSE infection. The challenge of other animal species with BSE-infected material, including sheep (37, 38, 50, 52) and mice (60), for example, indicates widespread distribution of infectivity and PrPSc in the LRS. A recent study suggests that the reduced involvement of the LRS in cattle may be a result of the form of PrP that is expressed on follicular dendritic cells of the bovine immune system (92). This form of PrP may hinder the replication of PrPSc in the LRS of cattle.

The detection of infectivity in tonsil (10 months post challenge) using cattle bioassay and in bone marrow (38 months post challenge) using mouse bioassay would also indicate the involvement of the LRS in the pathogenesis of BSE in cattle. Infectivity levels may simply have been extremely low, and below the levels of detection with the methods used. Although tonsil infectivity precedes the detection of infectivity in the CNS, the role of tonsil in the pathway to the invasion of the CNS is uncertain. Under current EC legislation, tonsil is classified as SRM in cattle over 6 months of age in the UK and Portugal and over 12 months in other EC member states.

Periodic detection of infectivity in the distal ileum (99) of BSE-challenged cattle has also indicated the involvement of the Peyer’s patches of the LRS. Recent immunohistochemical studies appear to confirm the accumulation of PrPSc in the Peyer’s patches of the distal ileum of cattle exposed to experimental oral challenge with BSE (91). It is noteworthy, however, that mouse bioassay and immunohistological studies of cattle naturally infected with BSE have failed to detect any infectivity or PrPSc in the distal ileum (39, 91).

Management of the epidemic in the United Kingdom

The initial epidemiological findings were considered sufficiently strong to be able to identify appropriate measures for statutory control in 1988. The main pieces of legislation described and the dates of their implementation relate to GB. Measures in Northern Ireland mirror those in GB. The statutory control measures have focused on three main areas:

– disease notification and statutory powers to slaughter and dispose of suspected cases
– controls on the content of feed for ruminants
– controls on the removal and disposal of tissues judged likely to be infective and designated as specified bovine offal (SBO) (later amended to SRM).

To achieve full and effective control of any animal disease, statutory measures alone should not be considered sufficient. Such measures should be supported by an appropriate infrastructure to inform the public about the disease and the legislative requirements, to monitor the efficacy of the control measures and to provide proper enforcement of the measures.

Disease notification

Bovine spongiform encephalopathy was made statutorily notifiable in GB in June 1988, when it became a legal requirement to notify suspected cases of the disease to the competent veterinary authority. Later in the month, interim advice from the Southwood Committee recommended the destruction of the carcasses of affected cattle and legislation for a slaughter policy was then introduced. This was accompanied by further legislation that provided for the payment of compensation to farmers for affected animals. Experience in the UK has demonstrated the need for adequate compensation in order to encourage the continued reporting of suspected cases to the authorities.

The slaughter policy allowed for the seizure of carcasses of suspect animals and for their disposal under official supervision. It prevented the slaughter of animals suspected of being infected with BSE in normal abattoirs, reducing the chance of infectious tissue entering both the human and the ruminant food chains.

In the early stages of the epidemic, before BSE was made notifiable, information on the disease was distributed to cattle veterinarians to inform them of the signs of the disease and to
encourage voluntary notification of cases for the initial epidemiological study. With the introduction of the requirement for statutory notification in 1988, wider coverage was required, involving the veterinary profession as a whole as well as the farming community. Scientific publications, video presentations illustrating the disease and talks to local veterinary groups all helped to raise awareness throughout the profession. Veterinarians within the official Veterinary Services received particular attention and detailed written instructions were prepared. Articles in the farming press, information leaflets and talks to farming groups provided similar information to the farming community. Information was also targeted at abattoirs and markets. Since this was a novel disease it quickly assumed a high profile in the UK, aiding the dissemination of information. This high profile has been maintained throughout the epidemic and BSE has attracted a great deal of media attention, not only because of the novel aspects of the disease in animals but also because of the concerns about risks to human health.

Throughout much of the epidemic, the mandatory reporting of clinically suspect animals by owners or veterinarians was the only method available to detect cases of BSE. The effectiveness of such reporting has always been difficult to assess since there is still no suitable ante-mortem test to detect pre-clinical disease, and it is difficult to ascertain the extent of potential under-reporting of the disease or of lack of appreciation of the clinical signs of BSE. In the absence of any single indicator, it was necessary to construct the fullest possible picture of the disease to monitor its progress, and to use a number of surrogate indicators to assess the degree of reporting.

One means of monitoring disease notification was to look for suspect animals which may have gone unreported. Routine veterinary surveillance at premises such as markets and abattoirs included checks for possible BSE suspects, as did routine on-farm work such as tuberculin testing.

Regular statistics on the disease provide another means of monitoring. Given the extent of the epidemic, the UK has been in a unique position to build up a comprehensive epidemiological picture and to look for any unexpected changes in the pattern of disease. Regular statistics include both temporal and geographical information on the disease.

Figure 1 shows the number of farms with their first clinical case of BSE. This figure shows that, even in 2002, at this late stage in the epidemic, there are still farms experiencing their very first case of BSE. This indicates that the level of awareness is still high, even among owners who have never before experienced the disease.

The entire epidemic, plotted by month and year of clinical onset, is shown in Figure 2.

During the peak of the epidemic, in late 1992 and early 1993, up to 1,000 new suspected animals were placed under restriction each week. In a few cases, these restrictions were removed when clinical signs disappeared or when an alternative diagnosis was reached. However, around 96% of the restricted animals were slaughtered as suspects and subjected to laboratory examination. Disease was confirmed in around 85% of the slaughtered suspects. The epidemic has declined since the 1992 peak and, in 2002, the average number of animals placed under restriction is just over 17 per week and disease has been confirmed in only around 56% of suspects examined. The decline in the percentage of suspects confirmed by laboratory examination is shown in Figure 3. It can be seen that this decline has been most marked since 1999. This may be explained by the fact that the occurrence of other disease conditions which may appear similar to BSE will, presumably, remain at a constant level. Consequently, as cases of BSE become less common, they will make up a smaller percentage of the pool of clinical suspects.

In addition, there are some animals that are reported as possible suspect cases but these animals are subsequently considered not to have BSE by the examining official veterinarian. In such cases, where the official veterinarian is satisfied that the animal is not suffering from BSE, no restrictions are served but details of the animal are kept on file. Records of these cases for the last few years show that, although the number varies from month to month, the trend remains fairly steady.

Thus, more animals are being reported than are shown to be infected and the proportion of suspects testing positive for the presence of the disease is declining. In addition, the number of reported cases subsequently judged not to be BSE has remained relatively constant over the last few years. Taken together, these observations indicate that there is still a high level of awareness of BSE in the UK and that the reporting of suspects is still being conscientiously performed.
occurring in animals exported from the UK to European countries, which were members of the European Community in 1989, indicated that there may have been an incomplete ascertainment of cases of BSE in imported animals (80). Indeed, even within GB, a further analysis of data from two surveys of OTMS (Over Thirty Months Slaughter Scheme – introduced in 1996 to dispose of cattle over 30 months old and to prevent their entry into the human food chain) cattle, conducted in 1999 and 2000 (see below), indicated that the prevalence of infection may have been larger than that estimated by mathematical modelling (28).

The introduction of systematic surveillance programmes for BSE has provided another means of estimating the occurrence of disease, independently of the statutory reporting of suspects. In the UK, surveys of a sample of animals slaughtered under the OTMS Scheme were conducted in 1999 and 2000, using histopathology and immunohistochemistry as the main diagnostic methods. As a result of the prolonged incubation period for BSE, the surveys were aimed at animals aged five years and over, to give the best chance of detecting the disease. Almost 4,000 animals were examined in the 1999 survey and almost 10,000 in the year 2000. A prevalence of 0.45% was found in 1999 and 0.42% in 2000.

Nevertheless, it must be accepted that, with a disease such as BSE, in which the early signs may be very variable and may be mistaken for signs of other chronic conditions, it is inevitable that some early cases may not be detected. This is especially the case because the disease occurs at a low within-herd incidence and frequently as a single case. Therefore, animals with chronic conditions such as lameness or loss of condition may be culled before clearer signs of BSE become apparent. There will, therefore, be more cases than are officially reported.

This is likely to be a problem wherever the disease occurs and perhaps especially so where it has appeared only recently, and at a much lower incidence than in the UK, as is the case for all other affected countries. An estimation of the risk of BSE occurring in animals exported from the UK to European countries, which were members of the European Community in 1989, indicated that there may have been an incomplete ascertainment of cases of BSE in imported animals (80). Indeed, even within GB, a further analysis of data from two surveys of OTMS (Over Thirty Months Slaughter Scheme – introduced in 1996 to dispose of cattle over 30 months old and to prevent their entry into the human food chain) cattle, conducted in 1999 and 2000 (see below), indicated that the prevalence of infection may have been larger than that estimated by mathematical modelling (28).

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In 1999, Switzerland introduced a programme of systematic targeted sampling using the Prionics Western blot as a diagnostic screening test (22). The surveillance compared the occurrence of disease in reported clinical suspects with that found by active surveillance in defined populations. These
populations included animals over 24 months of age which were disposed of as fallen stock, presented for emergency slaughter or presented for normal slaughter. The results of the first nine months of testing indicated that the targeted surveillance identified a considerable number of BSE cases in the late stages of incubation or with clinical disease. In particular, a high number of cases was detected among animals disposed of as fallen stock. Of the 18 additional cases detected, 12 were found in fallen stock, four in animals presented for emergency slaughter and two in animals presented for normal slaughter. A retrospective investigation of all 18 cases revealed that most had displayed some signs of distress, non-specific disease or loss of productivity. This highlights the fact that typical signs are not always expressed by animals in the clinical phase of the disease, especially in the early clinical stages, and emphasises the difficulty of identifying all clinical cases where the incidence is very low.

A subsequent publication, based on the same survey and also on a similar study in the following year (23), indicated that the likelihood of detecting BSE cases in 1999 and 2000 was at least 40 times higher in the risk categories of emergency slaughtered cattle and fallen stock, when compared with the likelihood of detecting BSE through the reporting of clinical suspects. It is unlikely that this relative risk will be constant across all affected countries, but the results stress the benefits of targeted surveillance.

The EC surveillance programmes introduced in 2001 (34) have shown that, even in the UK where there is a high level of awareness, a significant number of additional cases have been revealed by targeted surveillance. Figures for the whole of the EC from January to October 2002 are shown in Table II. The number of cases detected in the UK by active surveillance during this period was 513. Of these cases, 502 were sampled as risk category animals, which includes animals presented as fallen stock, subjected to emergency slaughter or found to be sick at ante-mortem inspection. The eleven other cases were identified among animals over 30 months of age presented for normal slaughter (i.e. slaughter via the OTMS Scheme – not for human consumption). These 513 cases identified by active surveillance may be compared with the 416 positive cases detected from 769 clinical suspects (19 results pending).

Table II
Bovine spongiform encephalopathy (BSE) testing in the European Community: cumulative results from January to October 2002

<table>
<thead>
<tr>
<th>Member country</th>
<th>Number of adult cattle (millions)</th>
<th>Reported animals suspected of having BSE</th>
<th>Risk category animals (not reported)</th>
<th>Healthy animals</th>
<th>Animals slaughtered in association with BSE cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No.</td>
<td>Tested positive</td>
<td>Results not yet known</td>
<td>Total No.</td>
<td>Tested positive</td>
</tr>
<tr>
<td>Belgium</td>
<td>1.5</td>
<td>252</td>
<td>3</td>
<td>202</td>
<td>14</td>
</tr>
<tr>
<td>Denmark</td>
<td>0.9</td>
<td>30</td>
<td>0</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>Germany</td>
<td>6.3</td>
<td>191</td>
<td>7</td>
<td>184</td>
<td>44</td>
</tr>
<tr>
<td>Greece</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>3.4</td>
<td>52</td>
<td>14</td>
<td>38</td>
<td>16</td>
</tr>
<tr>
<td>France</td>
<td>11.2</td>
<td>180</td>
<td>39</td>
<td>141</td>
<td>66</td>
</tr>
<tr>
<td>Ireland</td>
<td>3.6</td>
<td>445</td>
<td>90</td>
<td>355</td>
<td>90</td>
</tr>
<tr>
<td>Italy</td>
<td>3.4</td>
<td>88</td>
<td>0</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>0.1</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1.7</td>
<td>31</td>
<td>1</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Austria</td>
<td>1.0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Portugal</td>
<td>0.8</td>
<td>138</td>
<td>19</td>
<td>119</td>
<td>34</td>
</tr>
<tr>
<td>Finland</td>
<td>0.4</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sweden</td>
<td>0.7</td>
<td>33</td>
<td>0</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>5.0</td>
<td>769</td>
<td>416</td>
<td>353</td>
<td>152</td>
</tr>
<tr>
<td>Total</td>
<td>40.4</td>
<td>2,230</td>
<td>589</td>
<td>1,034</td>
<td>368</td>
</tr>
</tbody>
</table>

a) Source: Eurostat
b) Animals reported as being suspected clinical cases of BSE
c) Animals found dead on the farm, emergency slaughtered animals, animals sent for normal slaughter but found to be sick at ante-mortem inspection
d) Healthy animals subject to normal slaughter
e) Animals from birth and rearing cohorts, feed cohorts, offspring of BSE cases, animals from herds with BSE
f) Until the end of September
g) Great Britain and Northern Ireland

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These observations highlight the importance of achieving and maintaining a high level of awareness of BSE and of ensuring that the disease is fully considered in a differential diagnosis. Levels of compensation and policy decisions for action in herds which test positive for the presence of the disease should be such that they do not discourage the reporting of suspected cases. It may be argued that a policy of culling the entire herd when a BSE case is confirmed discourages the reporting of suspects. In Switzerland, when the national policy changed from whole herd to birth cohort culling, the notification of suspects increased (31).

**Feed controls**

The findings of the initial epidemiological study implicated the use of MBM as the likely vehicle of infection. However, the origin of BSE (see ‘Origin of infection’, above), it was evident that the infection was being recycled and amplified in the form of carcass waste from infected cattle. This waste was being processed into MBM which was then incorporated into commercial compound cattle feed. The first legislation to prevent the recycling of infection was introduced in July 1988 and prohibited the feeding of ruminant protein to ruminant animals. Subsequent legislation has expanded and tightened these controls, as circumstances have required. However, preventing infected MBM from entering cattle feed remains central to the control of the disease.

The dissemination of information on these feed controls was greatly assisted by close liaison with the feed manufacturers and their UK trade association. In addition, when the routine testing of feed began, written instructions were prepared for the official Veterinary Services who conducted the sampling programme. When the feed recall scheme was introduced in 1996 (see below), letters were sent out to farmers, feed mills and feed merchants in order to reach as many locations as possible where feed was stored.

At the introduction of the July 1988 feed ban, no direct means of monitoring was available. A technique to detect and to determine the species of origin of heat-stable proteins in rendered material was subsequently developed and a suitable enzyme-linked immunosorbent assay (ELISA) was introduced in 1994.

The effects of the 1988 legislation can be seen in the graphs at Figures 4a and 4b. Confirmed cases of BSE are plotted by the month and year of birth of the affected animals. Since exposure leading to infection appears to occur mainly in calfhood (106), the graphs reflect the levels of exposure to infection encountered throughout the epidemic.

The cyclical appearance of the graphs is a consequence of the seasonal calving pattern in UK cattle. An exponential rise in exposure is apparent up to the end of 1987. The introduction of disease control measures in 1988, in particular the feed ban, resulted in a 67% reduction in the hazard of contracting the disease for animals born in the first 12 months after these measures were introduced (85). Stevenson et al. also examined the effect of the SBO ban of 1990. The hazard of the disease for those born in the first 12 months after its introduction was reduced by a further 46%.

Although the incidence of BSE began to decline in 1993, it became evident in 1991 that confirmed cases of BSE were occurring in animals born after the 1988 feed ban (born after the ban or BAB), indicating continuing exposure to infection. The occurrence of these cases gave rise to much speculation about the transmission of BSE between cattle, either maternally or horizontally. When sufficient BAB cases had accumulated, a case-control study was initiated in 1993 to investigate a maternal risk factor and the possibility of horizontal transmission (47). The results indicated that neither maternal nor horizontal transmission could account for the majority of cases in animals born after the feed ban.

In-depth studies of affected animals born in the second half of 1988 indicated that they were at risk from feedstuffs in the food chain or on-farm which had been manufactured before the ban and therefore contained ruminant protein. In the case of animals born after this time, the reasons for the continuing, reduced level of feedborne exposure emerged after analysing the geographical variation in incidence. The most important finding was the increase in the proportional occurrence of BSE in animals born after the feed ban in northern and eastern regions of England. These regions contain a substantial proportion of the pig and poultry populations of the UK. The diets of these species were unaffected by the 1988 feed ban and an analysis by county revealed a 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. A comparison between Figure 5 and Figure 6 highlights the enhanced risk in the northern and eastern regions of England for the occurrence of BAB cases. This is discussed further in the section on cases confirmed in animals born after the reinforced feed ban.

Subsequent investigations, including the use of the newly introduced ELISA to detect species-specific, heat-stable protein, revealed the reason for continuing feedborne infection. Although there was no evidence of any significant deliberate contravention of the feed ban at the mills investigated, it was apparent that there had been accidental cross-contamination of cattle feed with ruminant protein, which was quite legitimately present in the feed mills for use in pig and poultry rations. There were several points at which this cross-contamination could occur. In particular, the use of shared intake pits for ingredients meant that the remnants of deliveries of MBM could be inadvertently left in augurs and on conveyor belts which were subsequently used to transport ingredients for cattle rations. Assumptions about the efficacy of flushing the
production lines were found to be overly optimistic, particularly given the evidence that the effective infectious dose was one gram or less (see ‘Risk of transmission of transmissible spongiform encephalopathies’, above). Other points of possible cross-contamination included spillage of pig or poultry rations in delivery lorries and the accidental feeding of pig or poultry rations to cattle, and in particular to calves.

In view of the difficulty of preventing cross-contamination of cattle feed which was produced in the same mills as feed for...
other livestock, further legislation was introduced in 1996. This legislation prohibited the use of mammalian MBM in all livestock feed. The introduction of this legislation was followed by a scheme to recall and destroy earlier stocks of feed remaining on farms, at merchants or in mills. The scheme was also accompanied by the thorough cleaning and disinfection of storage facilities and production lines to remove any residual traces of mammalian MBM. This exercise was completed in July 1996.

In 1996 the national programme of feed testing was extended to include up to 20,000 samples per year. This programme remains a key control measure for BSE.

One consequence of the total ban on the use of mammalian MBM in any livestock feed, and of the additional material produced by the OTMS Scheme, was the problem of disposing of MBM. This has necessitated the introduction of controls on MBM handling and disposal to prevent its inclusion, either accidentally or deliberately, into livestock feed.

This series of events illustrates the major challenge of controlling the feedborne source of BSE. The initial conclusions on the origin of infection remained valid but additional legislation was necessary to ensure more comprehensive protection of cattle in the light of the low dose required to infect animals and the ample opportunities for accidental cross-contamination at feed mills.

**Specified bovine offal (specified risk materials)**

The restriction on the use of potentially infected tissues from cattle carcasses in both human and animal food is an important area for statutory controls. As indicated earlier, the original assessment of potentially infected tissues was based mainly on studies of the distribution of infectivity in natural scrapie, while subsequent evidence on the pathogenesis of BSE in cattle led to a refining of the legislation. Risk tissues were originally referred to as SBO but were later re-designated as SRM. Initial controls in the UK were introduced as a public health measure but later extended to all animal feed, following the realisation that other species were potentially at risk of infection. In particular, the disease had been transmitted experimentally to pigs by the multiple parenteral inoculation of BSE-infected brains (19). Although this could not be considered either a natural or likely means of transmission, the possibility that infection could occur in other species was established. Furthermore, the first case of a spongiform encephalopathy in a domestic cat had been confirmed in the same year.
From April 1995, the government Meat Hygiene Service assumed responsibility for abattoir supervision in GB. The service produced its own detailed operations manual, which included comprehensive information on the removal of SBO/SRM. A video presentation was also prepared. Guidance notes were prepared for local authorities who are the enforcement agencies for the legislation. Written instructions were prepared for official Veterinary Services and a network of regional specialist veterinary staff were trained.

During the early 1990s, the EC, the UK Ministry of Agriculture, Fisheries and Food 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 in 1994 and indicated that the two systems used in the UK were ineffective (90). Rendering plants using these systems had to either change to another system or add a process with an acceptable time and temperature treatment. These changes began in January 1995.

During 1994 and into 1995, there was increasing evidence that cattle were still being exposed to the feedborne source, albeit at a much reduced level (47). In addition, attempts to reconcile the expected weights of SBO from carcasses at abattoirs suggested that not all of the proscribed material was being removed.

As a result, further measures were introduced, including the requirement to stain SBO with a solution of Patent Blue V. This dye resisted the rendering process and was detectable by mass spectrometry. In addition, more stringent requirements were introduced for record-keeping in relation to the removal of SBO/SRM.

It also became apparent that the removal of bovine spinal cord at slaughter was not fully effective and supervision of this process was increased significantly during 1995. Moreover, further measures were introduced at the end of the year, prohibiting the use of bovine vertebral column in the manufacture of mechanically recovered meat (MRM). Although mainly public health controls, these measures did help to reduce potential recycling of infected material in animal feed.

The changes in SBO legislation did not substantially alter the thrust of the measures but were a response to the apparently incomplete compliance with those earlier controls. This was a further illustration of the need for measures which can be clearly monitored and enforced.

The decline of the epidemic

Figure 2 illustrates the continuing decline of the epidemic. The conclusions of the initial epidemiological study still appear to be valid and the principal control measures remain centred on the prevention of feedborne exposure. Changes to the legislation have been aimed at improving compliance and have not introduced any fundamental modifications in approach.

The shift in the age at clinical onset gives an indication of the efficacy of control measures. The age at clinical onset for the whole epidemic is shown in Figure 7 and it can be seen that the peak is five years. A similar graph for cases confirmed in 2002 in Figure 8 shows that the peak age of clinical onset has increased to seven years, with a substantial number of cattle showing disease at eight years.

![Fig. 7](image-url)

**Fig. 7**
Age of cattle at clinical onset of bovine spongiform encephalopathy for confirmed cases in Great Britain between 1988 and 2002

![Fig. 8](image-url)

**Fig. 8**
Age of cattle at clinical onset of bovine spongiform encephalopathy for confirmed cases in Great Britain in 2002

Given the variability of the incubation period, this increase in the age at clinical onset suggests that exposure to infection has decreased and that most animals which are now expressing disease were infected before 1996. The graph at Figure 4b also suggests a substantial decrease in exposure from 1995 onwards.

In recent years, the GB BSE epidemic has been modelled in some detail to provide estimates of the future trend. Regular predictions have been produced by the model constructed by
the Veterinary Laboratories Agency. Until the year 2000, this model had given an accurate prediction of the number of cases which would present as clinical suspects. As shown in Table III, the actual number of cases had been within the 95% limits, although towards the upper level.

### Table III

Predictions produced by the Veterinary Laboratories Agency model of the number of animals per year which would present as suspected clinical cases of BSE in Great Britain (updated October 2001)

<table>
<thead>
<tr>
<th>Year</th>
<th>Central estimate of cases</th>
<th>Lower level of estimate</th>
<th>Upper level of estimate</th>
<th>Actual confirmed cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>2,083</td>
<td>1,774</td>
<td>2,392</td>
<td>2,254</td>
</tr>
<tr>
<td>2000</td>
<td>1,188</td>
<td>956</td>
<td>1,420</td>
<td>1,311</td>
</tr>
<tr>
<td>2001</td>
<td>512</td>
<td>360</td>
<td>664</td>
<td>781</td>
</tr>
<tr>
<td>2002</td>
<td>183</td>
<td>92</td>
<td>274</td>
<td>450</td>
</tr>
<tr>
<td>2003</td>
<td>61</td>
<td>9</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>19</td>
<td>0</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

However, it can be seen that the predictions for 2001 markedly underestimated the actual figure. This is considered to be an effect of the serious epidemic of foot and mouth disease (FMD) in the UK during that year. One of the consequences of the FMD epidemic was the suspension of the OTMS Scheme for several months of the year. As a result of this suspension, it is estimated that some 400,000 older cattle, which should have been disposed of through the scheme, remained on farms. The BSE model relies on a stable population structure and the increase in the number of older animals in the national herd has resulted in more BSE cases than predicted. It is uncertain how long this perturbation will last but, even when it has disappeared, it will be impossible to predict the tail of the epidemic or when the last case will occur, because of the protracted incubation period. Cases with a long incubation period could occur well into the future.

### Cases confirmed in animals born after the reinforced feed ban

Following the 1996 ban on the use of mammalian MBM in all livestock feed, it was assumed that cross-contamination of cattle feed could no longer occur. Furthermore, the subsequent recall of feed already produced should have prevented the use of old feed manufactured before the introduction of the ban. New legislation introduced on 1 August 1996 included a prohibition on the storage of mammalian MBM on premises where livestock feeds were kept. Therefore, calves born after 1 August 1996 should not have been exposed to feedborne infection.

The appearance of a small number of confirmed BSE cases born after 1 August 1996 is, therefore, highly significant for the UK. At present there are 33 such animals: four in Northern Ireland and 29 in GB. Of the 29 in GB, one was imported at the age of two years but the others appear to have been born in GB. The dates of birth of these animals range from August 1996 to July 1998 in GB and from September 1996 to May 1999 in Northern Ireland.

Each of these cases has been investigated in great detail in an attempt to identify a possible source of infection. These investigations include the gathering of epidemiological data on the natal and ultimate herd through the standard questionnaire used throughout the epidemic (108), supplemented by further questions considered relevant to these late-born cases.

A preliminary analysis of the first 16 cases has been published (105). The dates of birth of these cattle ranged from August 1996 to February 1998.

Of the 16 cases, eight were detected as clinical suspects and eight by active surveillance. Seven surveillance cases were emergency (casualty) slaughter animals and one was a healthy animal slaughtered in the OTMS Scheme. Although this is a small number of cases, some tentative conclusions can be drawn.

These cases have an increased age of onset of disease compared with that found in previous birth cohorts. The mean age at clinical onset or at slaughter was 57 months (range 46 to 66 months). This compares with 43 months (range 36 to 49 months) for the first 20 confirmed cases in the previous 12-month birth cohort (August 1 1995 to July 31 1996). The prolonged incubation period may be due to a reduced mean dose of exposure.

The distribution of cases between dairy and beef suckler herds was similar to that seen in the epidemic as a whole, with 15 cases in dairy herds and one in a beef suckler cow. However, the geographical distribution of the cases differs from that observed during the rest of the epidemic. Spatial analysis of the cases born after the 1988 feed ban revealed an increasing incidence towards the eastern part of GB and the major risk factor identified was the ratio of pigs to cattle (86, 104). The distribution for the major part of the epidemic, i.e. those animals born before the initial feed ban of 1988, indicated that the risk of a herd having an affected animal was greatest in the southern part of England where there was a greater risk of contaminated MBM. The geographical distribution of these 16 late-born cases is consistent with the major risk factor being the number of cattle herds per county. This suggests a random risk, consistent with a widespread distribution of a low risk of exposure.

There is no indication that these cases are the result of maternal transmission. None of the dams of the cases, or any other offspring of the dams, has developed clinical signs of BSE. Indeed, on five of the premises, the dam was still alive at the time the case was slaughtered. This is consistent with the epidemiological findings from the epidemic, which indicate
that the observed number of cases born after 31 July 1996 is considerably fewer than the risk estimated from the original cohort study would have suggested (111).

The initial conclusion is that these cases appear to be a third epidemiologically distinct series of the BSE epidemic in GB (the first series being those cases born before the initial feed ban of 1988 and the second being the BAB cases born afterwards). There is some evidence to suggest that cattle in this third series of cases were exposed to a reduced infectious dose and that the risk of infection is randomly distributed. There is no evidence of maternal transmission. Since four of the premises had not experienced previous cases of BSE, exposure from an environmental source is unlikely. A feedborne source therefore seems the most likely origin of infection. The possible contamination of UK-produced feed and of imported feed and ingredients is under investigation. Although no direct evidence of a source has been found to date, possibilities might include MBM legitimately used for the manufacture of pet food. Vegetable ingredients might also have become contaminated during passage through ports or ships which also handled MBM. The investigation of these cases continues.

Lessons and key conclusions

There are some important lessons to be learned from the experience of the UK in managing the BSE epidemic.

The principal disease control measures introduced after the initial epidemiological study remain the same today. The control of the cycle of re-infection via feed contamination appears simple, but the implementation of effective measures has proved to be more difficult than anticipated.

Furthermore, because the incubation period of the disease is so long, the effectiveness of control measures is difficult to assess at an early stage.

The key conclusions, on which the control of BSE depends, may be summarized as follows:

– the infectious dose is very small and cross-contamination is difficult to avoid
– legislation to control the disease must be accompanied by widespread dissemination of information and the effects of the legislation must be carefully monitored
– disease control policies should not discourage the reporting of clinical suspected cases
– some degree of active surveillance is necessary in order to assess the full extent of infection.

Acknowledgements

The authors would like to thank J. Wilesmith, D. Matthews and P. Soul for their helpful comments on this manuscript.

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L'encéphalopathie spongiforme bovine

M.J. Prince, J.A. Bailey, P.R. Barrowman, K.J. Bishop, G.R. Campbell & J.M. Wood

Résumé

Les premières études épidémiologiques ont révélé que l'encéphalopathie spongiforme bovine (ESB) était une infection alimentaire associée à la farine de viande et d'os (FVO) présente dans les aliments pour animaux. L'infection pourrait trouver son origine dans la tremblante du mouton, dans une mutation génétique survenue spontanément chez des bovins ou dans l'encéphalopathie subaiguë spongiforme transmissible (ESST) d'une autre espèce de mammifères. Les recherches sur le risque de transmission de cette maladie ont évidemment tenté de préciser les matières à risque et leurs niveaux d' infectivité, la nature et la solidité des barrières d'espèces, la dose infectante, la voie de transmission, la souche de l'agent causal ainsi que le génotype des animaux à risque. La détermination des niveaux d'infection des tissus de bovins a contribué à l'élimination des matières à risque des chaînes alimentaires humaine et animale.
Si la pérennité des foyers ne peut probablement pas être assurée par la seule transmission maternelle, les risques encourus par la descendance d’animaux malades sembleraient plus élevés à la suite d’une forte exposition à des aliments contaminés. Les études réalisées sur le transfert d’embryons ont permis de conclure à l’absence de transmission de l’infection à l’occasion de ce transfert. Les mesures prophylactiques, bien que simples en apparence, ont parfois été difficiles à mettre en œuvre et à évaluer. Cette situation a nécessité l’élargissement du champ des interdictions, une mise en œuvre plus contraignante et une vérification minutieuse de leur degré d’application.

**Mots-clés**


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**Encefalopatía espongiforme bovina**

M.J. Prince, J.A. Bailey, P.R. Barrowman, K.J. Bishop, G.R. Campbell & J.M. Wood

**Resumen**

Ya desde los primeros estudios epidemiológicos pudo determinarse que la encefalopatía espongiforme bovina (EEB) era una enfermedad transmitida por vía alimentaria y asociada a la ingesta de harinas de carne y huesos presentes en los piensos para animales. La infección podría tener su origen en el prurigo lumbar de los ovinos, una mutación genética espontánea ocurrida en el ganado vacuno o una encefalopatía espongiforme transmisible (TSE) que afecte a otra especie de mamífero. Las labores experimentales sobre el riesgo de transmisión se han orientado necesariamente hacia la determinación de los productos de riesgo y su nivel de infectividad, la naturaleza y solidez de las barreras interespecíficas, la dosis infectiva, la vía de infección, la cepa de agente etiológico y el genotipo de los animales en situación de riesgo. La cuantificación del nivel de infección en tejidos bovinos ha ayudado a retirar productos peligrosos de la cadena de alimentación tanto humana como animal. Es poco probable que un brote pueda perpetuarse sólo por transmisión por vía materna, aunque los descendientes de ejemplares clínicamente enfermos parecen más propensos a la infección en el caso de índices elevados de exposición por vía alimentaria. Los estudios de transferencias de embriones no han puesto de manifiesto que este proceder sea una vía de contagio. Aunque las medidas de control parecían sencillas, a veces ha resultado difícil aplicarlas y verificar el grado de observancia. Ello ha exigido imponer nuevas prohibiciones, adoptar medidas energéticas y controlar exhaustivamente los niveles de cumplimiento.

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