Bovine spongiform encephalopathy: the causal role of ruminant-derived protein in cattle diets

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Summary
Although bovine spongiform encephalopathy (BSE) has occurred in other European countries, the major epidemic has been in the United Kingdom (UK), where there have been more than 163,000 cases so far. BSE has been linked to the practice of feeding meat-and-bone meal (MBM), putatively contaminated with scrapie agent, to cattle. A ban on the feeding of MBM to ruminants in the UK has resulted in a significant decline in the number of reported cases. It is considered that BSE in other European countries probably originated through the use of British MBM in the diets of cattle in these affected countries. Recently, in the UK, a new variant form of Creutzfeldt-Jakob disease in humans has been discovered, which does not appear to have occurred before the advent of BSE. It may have been caused by BSE agent, possibly as a consequence of dietary exposure. The use of MBM in the diets of any livestock species has now been prohibited in the UK.

Keywords

Introduction
Bovine spongiform encephalopathy (BSE) belongs to a group of unusual transmissible degenerative encephalopathies (TDEs) of mammals, which are invariably fatal. The other principal TDEs are scrapie, which commonly affects sheep, and the rarer Creutzfeldt-Jakob disease (CJD) of humans. Although the precise molecular nature of the causal agents has not yet been determined, it is evident that they are not conventional micro-organisms. For example, an aberrant form of the PrP protein of the host appears to be an important component. As a consequence of infection by TDE agents, there is a change in the structure of PrP protein in the host, accompanied by a relative resistance of this modified protein to degradation by proteolytic enzymes. The resulting accumulation of modified PrP, particularly within the central nervous system, is associated with progressive neurological dysfunction which leads to death. Infected tissue from affected individuals can transmit these diseases within, and between, species. It would seem that the abnormal form of the PrP protein needs to be accompanied by an informational molecule, such as a nucleic acid (although none has yet been detected), to explain the stable, heritable strain characteristics, which can persist even after these agents have been passaged between species which have molecular differences in their PrP genes. TDE agents are relatively resistant to inactivation by procedures which are effective with conventional micro-organisms (4, 25, 43).

The first sporadic cases of BSE are thought to have occurred in the United Kingdom (UK) during 1985 (47). By October 1996, the disease had affected more than 163,000 British cattle, and had also occurred to a much lesser extent in the indigenous cattle populations of Ireland, France, Portugal and Switzerland. Epidemiological studies have shown that BSE in the UK was associated with the inclusion of meat-and-bone meal (MBM) as a protein supplement in cattle diets (51). MBM is manufactured by the rendering industry, principally from ruminant-derived tissues obtained from abattoirs. The working hypothesis for the emergence of BSE in the UK is that a combination of factors which prevailed in the late 1970s and early 1980s, which will be discussed, permitted the survival of sufficient scrapie infectivity in MBM to represent an effective oral challenge for cattle, resulting in sporadic cases of BSE. Once established in cattle, the disease was perpetuated by the
As BSE is a relatively new disease, and there is uncertainty regarding its origin, there has been international concern that it might affect indigenous cattle in countries other than the UK. In the other countries within which BSE has occurred in indigenous cattle, it is likely that the disease was introduced through the use of MBM imported from the UK in their cattle diets.

By 1989, more than 7,000 cases of BSE were being confirmed annually in the UK, and the disease had become an increasing focus of domestic and international attention. This led to damaging restrictions on the export of live animals and meat products from the UK because of speculation that human health would be compromised by dietary exposure to BSE agent, although this was not supported generally by scientific opinion (12,37). Nevertheless, from August 1988, all suspect cases of BSE in the UK were slaughtered and destroyed to prevent animal or human consumption. From November 1989, as an additional precautionary measure, specified bovine offals (SBOs) from cattle older than six months were excluded from the human food chain on the basis of what was known about titres of infectivity in the tissues of scrapie-affected sheep and goats (21, 22). The excluded tissues were the brain, spinal cord, tonsil, thymus, spleen and intestine. As a result of experimental pathogenesis studies with BSE in cattle (49), the intestine and thymus from calves under six months of age were excluded later. The European Community (EC) banned the export of SBOs (and certain other bovine organs used to manufacture biopharmaceuticals) from the UK to Member Countries in March 1990. In July 1991, the British authorities prohibited the export of SBOs and protein derived from such SBOs to countries outside the EC, and from November 1991 it became illegal in the UK to use SBOs in fertilisers. From September 1990, SBOs were excluded from all MBM used to feed livestock. SBOs are now stained with Patent Blue V and then rendered in dedicated plants, the MBM being destroyed by incineration.

In July 1988 a ban on feeding ruminant-derived protein to ruminants was introduced in the UK, and the BSE epidemic started to decline during 1993 as a result. The delayed effect was simply a reflection of the long incubation period of the disease, which is approximately four to five years on average. The incidence of the disease in the UK is now falling rapidly. In addition to the 1988 ban on feeding ruminant-derived MBM to ruminants in the UK, there is now a ban throughout the European Union (EU) on feeding mammalian protein to ruminants, unless it can be demonstrated reliably that ruminant protein is being excluded (17).

As BSE is a relatively new disease, and there is uncertainty regarding its origin, there has been international concern that it might affect indigenous cattle in countries other than the UK, Ireland, France, Portugal and Switzerland, within which the disease is currently endemic. The earlier opinions that BSE would probably not compromise human health were supported by studies in which transgenic mice carrying the human PrP gene were found to be susceptible following injection with CJD agent, but not following injection of the agent of BSE (10). However, the situation has become more confused since the recent discovery in the UK of ten cases of a new variant form of CJD, which has occurred in people below the age of 42 (55). Not only is the occurrence of CJD uncommon in this relatively young age group, but the reported cases shared clinical and neurohistopathological features which were unlike those associated with CJD prior to the existence of BSE. The authors were forced to the conclusion that these cases may be causally linked to BSE, perhaps as a result of dietary exposure to the agent. Consequently, the sale or supply of mammalian-derived MBM for feeding any species of livestock was banned in the UK in March 1996. From August 1996 it became illegal in the UK to possess feed containing MBM, and there was a statutory obligation to clean equipment and premises that had been in contact with MBM. At the same time, measures were taken to recover and destroy MBM, or feed containing MBM, which was held by manufacturers or which had been distributed.

A further complication has been the demonstration not only that sheep become infected with BSE agent after experimental oral challenge, but also that infectivity is detectable in a wider range of tissues than in BSE-infected cattle (20). Given that MBM has been fed to sheep in the past, there is a possibility that BSE might be recirculating in sheep in the UK and elsewhere, disguised as scrapie. If BSE was caused originally by scrapie agent, its transmissibility to sheep should not necessarily be regarded as surprising. However, the unique phenotype of the BSE agent derived from cattle is preserved after transmission to sheep. As BSE has been linked circumstantially to the new variant of CJD, the persistence of the BSE phenotype in sheep has given rise to human health concerns. The question as to whether certain sheep tissues should now be destroyed in the same fashion as SBOs is being considered in the UK (1) and the EU (3).

The epidemiology of bovine spongiform encephalopathy

The main epidemic

The first clinical case of BSE is considered retrospectively to have occurred in England during April 1985 but this new disease was not recognised formally until November 1986 (47). As a result of the close similarity between the neurohistopathological lesions in affected cattle and those in sheep affected by scrapie it was suspected that the cattle had become infected with a scrapie-like agent. Cattle and sheep had commonly co-grazed without scrapie crossing the bovine species-barrier, suggesting that, if scrapie agent was the culprit, it had probably infected cattle by an indirect route.
The widespread geographical distribution of early contemporaneous cases was not indicative of a propagative epidemic emanating from a single, focal source, and a dietary origin was speculatively suggested (15, 33). Thorough and extensive epidemiological studies demonstrated the direct correlation between the occurrence of BSE and the inclusion of MBM, putatively infected with a scrapie-like agent, as a protein supplement in cattle diets (51, 52). This explained the much higher incidence in dairy cattle, which commonly received such supplements in their diets, compared with beef suckler cattle, which received much less or none at all.

By October 1996, BSE had affected more than 163,000 cattle in England, Scotland and Wales (Ministry of Agriculture, Fisheries and Food [MAFF], unpublished data). Although other countries have reported a few cases in imported cattle, these animals were probably infected originally in the UK and showed clinical signs only after export. However, given that 58,000 adult breeding cattle were exported from the UK between 1985 and 1990, it is considered that there should have been many more cases of BSE reported abroad (8). BSE has also been observed in the indigenous cattle populations of Ireland, France, Portugal and Switzerland. By October 1996, the numbers of indigenous cases in these countries were 140, 25, 44 and 224, respectively (MAFF, unpublished data). It has been postulated that these countries may have imported British MBM which was then fed to cattle. It is known that, as the price fell in 1989, the amount of MBM exported from the UK more than doubled, compared with the preceding year (7). The reason for the general uncertainty is that the arrangements for international marketing of MBM tend to obscure its national identity, but it has been recorded that approximately 15,000 tonnes of British MBM were imported by France during 1989 (7). The fact that BSE has, so far, been confined to Europe suggests that what was originally a British problem was disseminated within Europe as a result of feeding MBM of British origin to European cattle. At the same time, MBM from the UK was also being exported elsewhere for incorporation into animal feed, but mainly to countries where pigs and poultry were the more common recipients. For example, Thailand imported 6,200 tonnes during 1991 (7). It has been shown that neither pigs nor poultry are susceptible to BSE after experimental challenge by the oral route (11).

Recent data suggest that approximately 1% of the British cases of BSE may be attributable to maternal association (35). However, it is important to note that these conclusions were arrived at before the ‘maternal transmission’ study was finished. Until this study is complete, it is not possible to know to what extent maternally associated cases are occurring, or even whether they are occurring at all (2). Epidemiological studies suggest that the most important factor in initiating and perpetuating the disease was the practice of incorporating MBM into cattle feed (51). Even if vertical transmission occurred commonly, control of the epidemic would be delayed but not prevented (50).

Apart from producing disease in cattle, since 1985 the BSE agent is suspected or known to have caused disease in other species. BSE-like disease has been reported in domestic cats in the UK, and the strain type has been identical to that of BSE (6). BSE-like disease has also been observed in exotic captive carnivores and ruminants born in the UK, to confirmation in kudu and nyala that the strain type is identical to that for BSE agent (6). The number of affected domestic cats and exotic species is shown in Table I.

### Table I

<table>
<thead>
<tr>
<th>Species</th>
<th>Number affected</th>
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</thead>
<tbody>
<tr>
<td>Domestic cat (Felis catus)</td>
<td>76*</td>
</tr>
<tr>
<td>Ankole (Bos taurus)</td>
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<tr>
<td>Arabian oryx (Oryx leucoryx)</td>
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</tr>
<tr>
<td>Eland (Taurotragus oryx)</td>
<td>6</td>
</tr>
<tr>
<td>Gemsbok (Oryx gazella)</td>
<td>1</td>
</tr>
<tr>
<td>Kudu (Tragelaphus strepsiceros)</td>
<td>8*</td>
</tr>
<tr>
<td>Nyala (Tragelaphus angasii)</td>
<td>1*</td>
</tr>
<tr>
<td>Scimitar-horned oryx (Orx dama)</td>
<td>1</td>
</tr>
<tr>
<td>Cheetah (Acinonyx jubatus)</td>
<td>3</td>
</tr>
<tr>
<td>Ocelot (Felis pardalina)</td>
<td>2</td>
</tr>
<tr>
<td>Puma (Felis concolor)</td>
<td>3</td>
</tr>
<tr>
<td>Tiger (Panthera tigris)</td>
<td>1</td>
</tr>
</tbody>
</table>

* Causal agent confirmed to be of the same strain type as that found in cattle with bovine spongiform encephalopathy (either untested)

### Bovine spongiform encephalopathy in cattle born after the ban on feeding ruminant-derived protein

As the highest incidence of BSE has been in four- to five-year-old cattle, the statutory ban on feeding ruminant-derived protein to ruminants, introduced in the UK in July 1988, might have been expected to have had a detectable effect during 1992. Although the incidence of the disease was greater in 1992 than in 1991, the increase was less than in preceding years, and in 1993 the incidence started to decline. However, the 1988 ruminant feed ban did not incorporate any measures to seize and destroy MBM which was already in existence, and so significant opportunities for infection would have existed for some incaulculable time thereafter. As it happens, the maximum input of BSE infectivity into MBM would have been in the period just before the feed ban (52). Nonetheless, the reduction in the incidence of BSE in two-year-old animals in 1991 and 1992, and a declining incidence in three-year-olds in 1992, were the first indications that the ban was having an effect. However, the customary incidence in these age groups was too low for these changes to have had a noticeable impact on the epidemic generally (53, 54). By October 1996, BSE had...
occurred in around 28,000 animals born after the ban but a high proportion of these were born in the period immediately after the ruminant feed ban (MAFF, unpublished data). Nevertheless, a significant number of cases are considered to be attributable to cross-contamination in the mills, which produced feed for ruminants, pigs and poultry using the same equipment. SBOs were permitted to be used to manufacture MBM for pig and poultry feed until September 1990, when the SBO ban to protect non-ruminant species was introduced. The occurrence of the disease in an animal born in 1992 suggests that ruminant-derived protein may still have been occasionally finding its way into cattle diet in 1992, despite the statutory control measures. In view of the continuing significant decline in the incidence of BSE, this was obviously not a major problem but it was acknowledged that the elimination of any such loopholes would become increasingly important if the disease was to be eradicated. It has been ascertained recently that the statutory separation and disposal of SBOs have not always been as secure as anticipated, and so they could sometimes still have been used to manufacture MBM. Consequently, legislative control was tightened by the introduction of a new Order in August 1995. As a consequence of this Order, it became no longer permissible to remove bovine brain from the skull prior to rendering in the UK. Thus the whole head, excluding the tongue and head-meat, became an SBO. The current, and highly significant, rate of decline in the incidence of BSE in the UK indicates clearly that the ruminant protein feed ban introduced in 1988 has had a dramatic effect. This supports the conclusion from epidemiological studies that MBM has been the major source of infection. There has been only limited evidence of transmission related to maternal association, and it is likely that the occurrence of BSE in cattle born well after the initial feed ban has been associated principally with cross-contamination of cattle diet in mills, which also produced pig and poultry feed containing MBM. A within-herd case control study conducted in 1993 and 1994 indicated that neither maternal nor horizontal transmission could account for the vast majority of cases born after the feed ban (23). The development and use of an enzyme-linked immunosorbent assay (ELISA) for ruminant protein, and the new policies of MAFF for controlling SBO separation and processing, were designed to remedy any pre-existing weaknesses in cattle-feed manufacture, since exposure to the food-borne source appears to be the principal risk factor. ELISA testing of animal feed over an eighteen-month period showed three positive results for ruminant-derived protein out of 1,579 samples tested, but the possibility that these represented non-specific reactions could not be excluded (30). However, the recent ban on the use of MBM in the diet of any livestock species in the UK makes these measures largely irrelevant as far as animal health is concerned, provided that the legislation is obeyed and enforced. Assuming that MBM incorporating SBOs can now be completely excluded from cattle diets, some cases of BSE are still likely to occur until the first decade of the next century because of the possible occasional exposure to potentially contaminated food until 1995 (41). The situation would be somewhat different if horizontal transmission occurs. At present, there is no evidence for this, which suggests that, at worst, it is a rare occurrence. However, it will require a few years of complete freedom from BSE to be sure that this is not a complicating factor. Indeed, if it was, one could envisage a policy of slaughter of a limited number of complete herds being necessary in order to achieve eventual eradication. There is every indication at present that BSE can be eradicated in the UK, provided that there is strict adherence to the new regulations.

**Strain typing of bovine spongiform encephalopathy agent**

The incubation period of experimental scrapie in mice is controlled predominantly by the two alleles of the scrapie incubation period (Sinc) gene, which is homologous with the PrP gene. Individual strains of scrapie agent have different but precise incubation periods in mice of different Sinc genotypes. Each strain also produces a characteristic profile of vacuolar lesions in the brains of these Sinc genotypes. However, during primary transmission of natural scrapie from sheep to mice the incubation period patterns are variable, as are the patterns of neurohistological lesions (13), even within a single Sinc genotype (6). It is only after serial passage in mice that strains with defined and reproducible phenotypic characteristics emerge.

In contrast, all of the BSE-infected cow brains that have been injected into five genotypes of mice had incubation periods that were closely aligned within each genotype, but were significantly different between mouse strains of the same or different Sinc genotypes (6). The cow brains tested had been deliberately collected from unrelated cattle in different geographic locations between 1987 and 1990. This information, together with the fact that the lesion profiles in mouse brain were remarkably uniform, suggests that BSE is caused by a single strain of agent. This is supported further by the consistency of the topography of brain lesions in cattle with natural BSE (48). An unexpected and striking feature of the BSE agent is that it retains its characteristic phenotype, even after experimental passage through sheep, goats or pigs (6). Moreover, transmission of disease to mice from three domestic cats, a greater kudu and a nyala with spongiform encephalopathy resulted in the same phenotypic expression in mice (6). These findings provide strong evidence that the BSE-like disease seen in domestic cats and captive felids and ungulates since the start of the BSE epidemic has been caused by the same agent that affects cattle.

There is convincing evidence that Swiss BSE is linked to the British epidemic because, in contrast to the other countries
within which indigenous BSE has been reported, the Swiss commissioned studies to determine whether their BSE strain type corresponded with that in the UK. The Swiss strain type was identical to that of the unique strain type which has been shown to cause BSE in British cattle (5). This provides convincing evidence that the Swiss epidemic was caused by incorporating MBM of British origin into Swiss cattle feed. The same may be true for the other three countries outside the UK within which indigenous BSE has been reported, but this will remain unproven in the absence of strain typing.

The role of meat-and-bone meal in the aetiology of bovine spongiform encephalopathy

Having excluded all other possible aetiological factors, it was concluded that BSE was associated with the practice of feeding MBM to cattle (51). Among the factors relating to MBM production which might have contributed to the emergence of BSE, it was noted that there had been a significant shift from batch to continuous rendering in the UK. However, for such a change to have been significant, it would need to have occurred predominantly between the late 1970s and early 1980s to accord with the average five-year incubation period for BSE, and the occurrence of the first putative cases in 1985. It is assumed that incubation periods equate reasonably with age since the earliest and often only exposure to MBM is in calfhood (51). In fact, the most significant swing from batch to continuous rendering had already occurred by the late 1970s (52).

Another trend in British rendering practice, which was identified as having a potential association with the onset of BSE, was that there had been a widespread abandonment of the solvent extraction procedure (33). This process provided an additional yield of tallow by recovering residual fat from material which had already been rendered. In addition, low-fat MBM was obtained which traditionally attracted lower prices. However, a change in demand, which had earlier favoured MBM with a lower oil level, resulted in a shift away from solvent extraction in the UK (32). In 1975, approximately 65% of the MBM produced in the UK had been subjected to solvent extraction; by 1982 this proportion had decreased to 10% (52). It is this change over the timeframe described which is more convincingly linked to the emergence of BSE. However, the solvent extraction process does not appear to have had the potential for a high degree of inactivation of scrapie-like agents. Although there are no data for the solvents used in the rendering industry, organic solvents per se do not generally have any great effect on crude tissue homogenates containing these agents (39). However, it may be that inactivation by solvents was enhanced in the extraction process because they were heated (typically 60°C to 80°C for up to eight hours), even though exposure to these temperatures alone would cause no significant inactivation. Similarly, the subsequent treatment with dry heat to drive off the solvent (typically at 100°C for 30 min) would not be considered to be particularly effective (14, 45), unless there was a synergism associated with the presence of solvent. The final phase of production involved exposure to steam to evaporate residual solvent. Although steam provides a much more effective form of sterilisation than dry heat, the time/temperature ratios involved (typically 100°C for 30 min) were probably too low to provide significant inactivation (25, 38, 42, 43). However, it is acknowledged that the data available for thermal inactivation of scrapie and analogous agents are derived from experiments using homogenates of infected brain tissue, usually as a deliberate strategy to create a worst-case scenario in setting decontamination standards. In contrast, the end products from the solvent extraction process had a low fat content when compared with raw tissue; therefore the thermal treatments may have been more effective than anticipated because the presence of lipid impairs the efficiency of heat sterilisation. As the solvent extraction process had been applied to tissues that had already been subjected to heat during typical rendering procedures, it may be that this process provided a marginally increased degree of inactivation which was just sufficient to prevent the titre of surviving infectivity in MBM from being an effective oral challenge for cattle. A study is in progress to determine the effect of solvent extraction on BSE and scrapie agents.

It was also suggested that a further factor relevant to the occurrence of BSE was an increase in the sheep population in the UK during the late 1970s and early 1980s, accompanied by an increased number of scrapie cases (33). The national sheep population expanded significantly from 21.6 million in 1980 to 27.8 million in 1988 but this does not appear to have resulted in an increase over that period in the proportion of ovine tissues (15.3%), compared with other species used in rendering (52). In addition, although the number of cases does appear to have increased (34, 51, 56), there seems to have been no increase in the within-flock incidence (56), implying that the level of scrapie infectivity in rendered waste did not alter in the UK during the 1980s. However, this interpretation is probably incorrect because sheep heads and carcasses of casualty and condemned sheep were increasingly being incorporated (51), which could have increased the load of scrapie infectivity in raw materials for rendering.

The relationship between bovine spongiform encephalopathy and scrapie

Although the phenotypic characteristics of the single strain of agent which causes BSE are unlike those of any strain of scrapie agent identified to date (6), it is known that
inter-species transmission of scrapie can result in permanent changes in strain phenotype (26). Moreover, the existence of a variety of strains of scrapie agent is well established and these vary in their degree of thermostability (14, 25). It is possible that an uncommon but thermostable strain survived in MBM at a sufficiently high titre to have caused BSE (40).

The probability that BSE represents the transmission of the sheep disease, scrapie, to cattle via MBM was deduced from a thorough survey of potential aetiological factors, including the practice of co-grazing cattle and sheep (51). The possibility that BSE resulted from the sudden evolution of a strain of scrapie agent with enhanced virulence for bovines (even by passage in cattle), or a similar mutation in a pre-existing bovine agent, which had previously been 'silent', was excluded on the basis of the widespread geographical distribution of contemporaneous cases early in the epidemic (51). This was not indicative of a propagative epidemic emanating from a single focal source, and accorded with the knowledge that MBM was manufactured and distributed on a relatively parochial basis. There had been changes in rendering methods which, arguably, could have resulted in enhanced survival of scrapie agent, especially if the amount of scrapie-infected raw material had been increasing. Scrapie has been endemic in the UK for more than two centuries, and 30% of sheep in the EU are in the UK (European Renderers Association [EURA], unpublished data). These factors were considered to have changed over a time-scale which was synchronous with the first putative exposure of cattle to the causal agent of BSE (33).

As the pressure on the farming industry to produce cheap food increased, there was a trend towards the use of protein supplements in commercial or farm-produced feed for livestock. As a result of market fluctuation, this varied between the addition of vegetable-derived or animal-derived protein. However, during the 1980s there was a commercial advantage in using MBM as the protein supplement because of the escalating cost of soya and fishmeal. The inclusion rate of MBM in British animal feed rose from 1% to 12% over this period. By comparison, the inclusion rate in French cattle diets was typically 1.5% (7). These factors, together with the very high ratio of sheep to cattle in the UK (22 million to 12 million in 1980), were considered to have collectively created conditions under which the level of scrapie infectivity in MBM increased sufficiently for it to represent an effective oral challenge for cattle, and cause BSE.

There has always been a stigma associated with the occurrence of scrapie in sheep, and it is said that farmers with affected animals sometimes try to dispose of them before severe clinical signs develop, especially if they come from flocks that are valued as good commercial breeding stock. Consequently, it has always been difficult to ascertain the true level of scrapie in the sheep population of the UK (or elsewhere). In 1993, scrapie was made a notifiable disease throughout the EU. This legislation was introduced for reasons associated with trading, and not with any real expectation of revealing the true extent of scrapie within the EU. Indeed, if the rate of notification was taken as a true representation of the incidence of scrapie in the UK, one would conclude mistakenly that the disease was on the decline. For example, the number of cases notified in the UK during 1995 was 116 (31). However, in 1990 there was a need to obtain brains from scrapie-affected sheep in the UK for an experimental study. As an incentive, £15 per affected animal was offered to sheep-farmers (9). Within 22 months, 2,867 brains had been acquired.

From the time of the earliest exposure of cattle to effective dietary doses of infectivity, the only two surviving solvent extraction plants in the UK had been in Scotland. These plants were known to have manufactured a large proportion of the MBM used, at least in 1988, in Scotland (52). Given the low incidence of BSE in Scotland, and the fact that the reported incidence is inflated artificially through the known acquisition of (subclinically) BSE-affected cattle from England, it has been suggested that these facts endorse the hypothesis that the large-scale reduction of solvent extraction in the UK permitted the emergence of BSE (51). The incidence of scrapie in Scotland compared with in England is unknown. It is therefore impossible to assess whether the solvent extraction plants in Scotland had to contend with greater or lesser loads of scrapie infectivity than plants outside Scotland, which did not use solvent extraction. However, raw materials are procured on a relatively parochial basis, and the Scottish plants would undoubtedly have received much less BSE-affected bovine raw material than their English counterparts. It is considered that this recycling of BSE infectivity caused the expansion of the BSE epidemic before the introduction of the MBM feed ban.

Although the balance of evidence suggests that scrapie agent was the causal factor for BSE, it should be acknowledged that there is circumstantial evidence that the most recent outbreak of transmissible mink encephalopathy (a scrapie-like disease) in the United States of America (USA) resulted from feeding mink with bovine tissues (29). BSE has not been detected in the USA, despite active surveillance, but the possibility has to be entertained that BSE might occur spontaneously, but rarely, in bovines world-wide just as sporadic CJD appears to do in 1:10^6 of the human population.

**Manufacturing processes for mammalian-derived protein products**

**Meat-and-bone meal**

For centuries, rendering has been used to extract tallow from waste animal tissues for incorporation into products such as candles and soap. Rendering procedures are essentially
cooking processes which permit the melted fat to be collected as commercial tallow. In the 1920s, it was realised that the greaves (solids which remain after tallow has been extracted) contained valuable proteins which could be pulverised to produce MBM for incorporation into feedstuff for farmed animals. MBM is now manufactured world-wide by the rendering industry from animal tissue discarded by abattoirs. In 1988, British renderers processed approximately 1.5 million tonnes of animal waste, from which about 400,000 tonnes of protein meal and 170,000 tonnes of fat products were obtained. In the EC, over 9 million tonnes of raw materials were processed over the same period to produce approximately 2.5 million tonnes of meal and a million tonnes of fat products (27). The amounts of mammalian protein products manufactured in the EU during 1995 are shown in Table II.

### Table II

<table>
<thead>
<tr>
<th>Country</th>
<th>Meat-and-bone meal</th>
<th>Blood meal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>76,166</td>
<td>1,413</td>
<td>77,579</td>
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<tr>
<td>Belgium/Luxembourg</td>
<td>132,000</td>
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<td>Denmark</td>
<td>182,471</td>
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<td>Finland</td>
<td>24,000</td>
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<tr>
<td>France</td>
<td>606,100</td>
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<td><strong>2,940,676</strong></td>
<td><strong>167,609</strong></td>
<td><strong>3,108,285</strong></td>
</tr>
</tbody>
</table>

Although the standards for manufacturing MBM for feeding ruminants have been revised recently within the EU (18, 19), it is important to consider the processes which were in place when the BSE epidemic began. Rendering procedures traditionally used throughout the EU are almost entirely based upon the principle that the fat contained in animal waste tissue can be made fluid by heating, to enable it to be separated from the solids which can then be milled to provide MBM. In the cooking process, raw materials were heated by the metal surfaces of the cooking equipment which, in turn, were heated by hyperbaric steam. A more rapid heating of raw materials was achieved in some systems by adding them to tallow which had been pre-heated.

Cooking usually proceeded at atmospheric pressure until the water was driven off at 100°C; the temperature then increased, and the tallow was allowed to drain off or was removed by centrifugation. The target temperatures and the holding times varied according to the required physical quality of the end products and so that a satisfactory level of decontamination was achieved, at least for conventional micro-organisms. There was also considerable variation in the selected size of particle to which the starting material was crushed before cooking.

Exceptions to the above general procedure included low-temperature wet rendering, in which raw materials were subjected to temperatures lower than 100°C for just long enough to melt the fat. The water-laden, high-quality tallow was separated from the solid fraction by centrifugation or compression; the remaining solids contained sufficient residual fat and water to be cooked in a standard fashion. Another method facilitated the removal of water from raw materials by cooking under hypobaric conditions; this was both energy saving and produced high-quality tallow. The third exception was when the cooking vessel was sealed hermetically during processing and before heating; the production of steam from the raw material resulted in hyperbaric conditions in which such steam had an abundance of latent heat energy. This system had only been operated previously in countries such as Germany where, unlike the UK, renderers have a statutory obligation to dispose of high-risk carcasses, such as those from foot and mouth disease. However, as a consequence of experimental rendering studies with scrapie agent (46), this procedure will become the only one permitted in the EU for manufacturing MBM to feed animals. The required conditions will be a temperature of at least 133°C for 20 minutes at an absolute pressure of three bars (19).

A secondary process involving solvent extraction is sometimes applied to recover the tallow which remains in greaves after cooking and removal of the free-run tallow. Typically, greaves are perfused over a period of several hours with recirculating hot organic solvent. Solvents which are known to have been used are benzene, hexane, heptane, perchlorethylene, petroleum spirit and trichlorethylene. After residual fluid has been allowed to drain from the greaves, the tallow-rich solvent is boiled to drive off the solvent through a water-cooled condenser, leaving the tallow fraction. Solvent remaining in the processed greaves is evaporated off by two heating stages. In the first stage, the solid materials are heated in a steam-jacketed vessel from which vapourised solvent is vented off. In the final stage, any remaining solvent is stripped from the solids by exposure to live steam. The dried solids are milled to produce low-fat MBM. In exceptional cases, extraction is carried out on raw materials rather than greaves.

Solvent extraction was common in the UK, accounting for approximately 70% of MBM produced between the mid-1960s and mid-1970s, but had been largely abandoned for economic reasons by the early 1980s. In contrast, the process was still being used in the 1990s in other countries.
e.g. Belgium, France and Italy, for up to 50% of MBM manufacture (EURA, unpublished data).

**Blood meal**

Blood meal is manufactured throughout the world. Blood collected from abattoirs is coagulated by steam injection, the temperature reaching 90°C to 96°C. The protein-rich coagulum is separated by centrifugation, and then dried by one of two methods. In hot-air drying, a ten-minute exposure at up to 110°C is used. For contact drying, a maximum temperature of 130°C is achieved by the end of a maximum three-hour exposure to indirect heat. Although a large proportion of the blood used to prepare blood meal originates from ruminants, it is unlikely to have had any significant role in the BSE epidemic because it was fed only sporadically to high-performance dairy-cows. Furthermore, BSE infectivity has not been detectable in the blood of BSE-infected cattle (36).

**Experimental studies on rendering processes**

Although a number of studies had been carried out on heat inactivation of TDE agents, none had mimicked the conditions found in rendering (38). From surveys of rendering practices in the UK (52) and the EU (27), it was possible to define generically the range of temperatures and times used in the various manufacturing procedures, together with the average particle sizes of the crushed raw material used in the different processes. These data were used to design experimental, pilot-scale facsimiles of these production methods which were used to process raw materials spiked with BSE or scrapie agents, to determine whether infectivity could be detected in the end products. The amount of infectivity in the spiked raw materials was $10^{1.7} \text{ID}_{50}/\text{g}$ for BSE agent and $10^{3.1} \text{ID}_{50}/\text{g}$ for scrapie agent.

BSE infectivity was detectable in MBM produced by two types of procedure (44). One was a process in which cooking took place at atmospheric pressure over a period of 50 minutes, with the end temperature reaching either 112°C or 122°C. The other was conducted under vacuum with added pre-heated fat, in the form of tallow. Exposure times were either 10 or 40 minutes, and the final temperatures were 120°C and 121°C, respectively. As a result of these positive findings, the minimum conditions for rendering ruminant-derived material within the EU were revised (18). The processes which were shown by the above study to permit survival of BSE infectivity had been introduced in an increasing number of premises in the UK during the early and mid-1970s, and so cannot alone account for the appearance of BSE in the 1980s. From these studies, it was concluded that the effective exposure of cattle to the BSE agent had been the result of a two-stage process, involving the cumulative effect of major changes in rendering practices, which occurred firstly in the 1970s, and then in the early 1980s in the case of solvent extraction. More recent studies on the same rendering processes, using scrapie-spiked raw materials, have shown that MBM produced by all processes (except those involving exposure to pressurised steam) was infected (46). Consequently, the only procedure which will be permitted for processing mammalian raw material into MBM for incorporation into animal diets in the EU will involve processing either the raw materials or MBM in steam at a pressure of three bars and a temperature of 133°C for at least twenty minutes (19). In view of recent concern that the cases of new variant CJD in the UK may be causally linked with BSE through dietary exposure (55), the use of MBM in the diet of any farmed animal species in the UK has been prohibited.

The known relative resistance of scrapie agent to thermal inactivation casts doubt on the ability of any current rendering technique to achieve total inactivation of BSE or scrapie agents where there is a very high input level of infectivity. The scrapie-spiked rendering experiments did produce MBM with no detectable infectivity after hyperbaric processing at 133°C for 20 minutes, but it should be noted that hamster-passaged scrapie agent has survived autoclaving for 60 minutes at either 132°C (16, 24) or 134°C (43). However, the level of infectivity of the hamster-passaged agent was approximately a million-fold higher than that in the scrapie-spiked raw materials; the number of infectious doses present in the latter was considered to exceed the worst-case conditions which could prevail in everyday rendering.

In the scrapie-spiked rendering studies, infected greaves were also subjected to solvent extraction with hot heptane. After draining, the greaves were exposed to dry heat to drive off residual solvent. The level of infectivity in the rendered tissue, before solvent extraction, was low. However, infectivity was still recoverable after treatment with heptane, and heptane plus dry heat. This tends to confirm the view that solvent extraction systems probably have little inactivating effect on scrapie-like agents (38). However, further quantitative laboratory studies on solvent extraction systems are in progress, and should provide a more definitive answer.

The greater number of positive MBM samples in the scrapie-spiked experiments, in comparison with the BSE-spiked studies, may well be associated with the higher input titre but differences in thermostability between the two agents cannot be ruled out. As discussed earlier, there is uncertainty regarding the origin of BSE because no strain of scrapie with BSE-like properties has been isolated from sheep as yet. If, as has been postulated, the strain of scrapie that caused BSE originally is a minor but thermostable one, it may well have been selected from the mixture of strains in the spike to survive in a relatively purified state in the infected MBM. An alternative possibility is that the rendering process alters the phenotype of a relatively common strain of scrapie.
Future directions

One of the original aims of the experimental rendering studies was to identify procedures which could be used to manufacture MBM for incorporation into animal feed that was reliably free from TDE agents (28). Although the results of these studies have indicated that processes involving pressurised steam are effective under what might be considered to be worst-case conditions (46), there seems little likelihood at present that British or EU legislation will be revised to permit the use of ruminant-derived proteins in feedstuff for ruminants (or even other farmed species in the UK). This is understandable, given the catastrophic effect that the occurrence of BSE and new variant CJD have had on the beef trade, not only in the UK but elsewhere within the EU. There is also a public perception that cattle which were fed MBM containing animal-derived proteins had been forced to indulge in cannibalism, given that they are natural herbivores. In fact, it is not true that cattle do not normally consume animal protein; protozoa (which are animals) convert plant protein to animal protein in the bovine rumen, and are themselves digested within the bovine alimentary tract. There is also a hypothesis which argues that if animals of a particular species, regardless of whether they are carnivores, herbivores or omnivores, are forced to eat material derived from animal species that are not part of their normal diet, it should not necessarily be surprising if they succumb to disease because they have no natural resistance to pathogenic agents present in the food source. As a result of the occurrence of BSE, this argument has been targeted particularly at ruminants which were fed MBM, but this product has also been fed commonly to pigs and poultry as a useful protein supplement, without ill effect. However, if foreign protein can be used efficiently by the recipient species, and can be effectively freed from any harmful transmissible agents which might affect that recipient species, it seems illogical to suggest that this is unacceptable because it is ‘unnatural’, given that the simple practice of farming animals is ‘unnatural’ as far as the animals are concerned.

It is likely that the political and public perceptions relating to BSE will prevent legislators in the UK or the EU from considering any move in the near future to relax the present restrictions on the use of MBM as animal feed. It may be that they consider that the margins for error in the rendering studies (44, 46) are too small to take any chances. However, new rendering processes which may provide an even greater degree of reassurance are being assessed currently. If any of these can be shown to be even more reliable than the best of the currently used procedures, there may be a good case for allowing MBM to be once again fed to ruminants and (in the UK) other farmed species, in particular because of the lower risk factor arising from the exclusion of SBOs and, possibly, specified ovine tissues.

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Encéphalopathie spongiforme bovine : le rôle des protéines de ruminants dans le régime alimentaire des bovins

D.M. Taylor & S.L. Woodgate

Résumé

Même si l’encéphalopathie spongiforme bovine (bovine spongiform encephalopathy: BSE) s’est déclarée dans plusieurs pays européens, c’est au Royaume-Uni, où plus de 163 000 cas ont été enregistrés à ce jour, que l’épidémie a été la plus grave. La BSE a été imputée aux farines de viande et d’os, ajoutées aux rations alimentaires des bovins et suspectées d’être contaminées par l’agent de la tremblante du mouton. L’interdiction de ces farines dans l’alimentation des ruminants au Royaume-Uni a donné lieu à un recul sensible du nombre de cas signalés. On pense que, dans les autres pays européens, la BSE est probablement due à l’utilisation de farines de viande et d’os britanniques dans l’alimentation des bovins de ces pays. On a récemment découvert, au Royaume-Uni, une nouvelle
La forme de la maladie de Creutzfeldt-Jacob chez l’homme, qui ne semble pas avoir été observée avant l’apparition de la BSE. Elle pourrait être due à l’agent de la BSE, probablement à la suite d’une exposition alimentaire. Les farines de viande et d’os sont désormais interdites au Royaume-Uni dans l’alimentation de tout le bétail.

**Mots-clés**


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**Encefalopatía espongiforme bovina: el papel causal de los piensos proteicos derivados de rumiantes en la dieta del ganado vacuno**

D.M. Taylor & S.L. Woodgate

**Resumen**

Aunque se han dado casos de encefalopatía espongiforme bovina (*bovine spongiform encephalopathy: BSE*) en otros países de Europa, la epidemia de mayor amplitud es la que se ha desatado en el Reino Unido, donde hasta la fecha se han descrito más de 163,000 casos. La BSE ha sido vinculada a la práctica de alimentar al ganado con harinas de carne y huesos, supuestamente contaminadas con el agente causante del prurigo lumbar. La prohibición de alimentar a rumiantes con dichos piensos se ha traducido, en el Reino Unido, en un significativo declive del número de casos registrados. Se considera probable que los casos de BSE observados en otros países de Europa fueran causados por la introducción de harinas británicas en la dieta de los bovinos de estos países. Recientemente se ha descubierto en el Reino Unido una nueva variante de la enfermedad de Creutzfeldt-Jakob, enfermedad que afecta los humanos, al parecer no observada con anterioridad a la aparición de la BSE. El agente de la BSE, posiblemente vehiculado a través de la dieta, podría ser la causa de esta variante. El empleo de harinas de carne y huesos para la alimentación de cualquier tipo de animal de granja ha sido ahora prohibido en el Reino Unido.

**Palabras clave**

References


