1 Introduction

Creutzfeldt–Jakob disease (CJD) and bovine spongiform encephalopathy (BSE) belong to a distinct group of fatal transmissible degenerative encephalopathies (TDEs) of animals and humans (Table 1). The classical, sporadic form of CJD affects around one or two per million of the human population worldwide each year, causing death at a median age of around 66. However, it was reported in 1996 that 10 cases of a new variant form of CJD (vCJD) had been recently identified in relatively young individuals in Great Britain but not apparently elsewhere. This was of widespread interest because (a) the clinical signs of vCJD were unlike those of sporadic CJD, (b) the period of clinical disease before death was much longer than for sporadic CJD, (c) death occurred at a median age of 29, (d) the neurohistopathological features of vCJD were different from those in sporadic CJD, and (e) it was concluded that, in the absence of any other obvious cause, the most likely explanation for vCJD was dietary exposure to the agent that had caused a major epidemic of BSE in the UK. This epidemic began around 1985, peaked in 1993, and is currently in steep decline. The incidence of BSE in the UK cattle population has considerably exceeded that in any other BSE-affected country, with more than 177,000 cases having been confirmed by July 2000. By the same time, the number of confirmed vCJD cases in the UK had escalated to 75; three cases had also been identified in France, and one in Ireland. Until 2000, the rate of occurrence of vCJD had been relatively constant, which made it difficult to predict what the eventual scale of this human epidemic might be. However, by July 2000, the number of cases already observed in that year suggested that the incidence was rapidly increasing. This indicated that the eventual scale of the epidemic might be far from modest but that further time would have to elapse to determine whether or not the apparently escalating incidence would continue. Given that this chapter was completed in July 2000, no

### Table 1 The transmissible degenerative encephalopathies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Affected species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie</td>
<td>Sheep, goats, moufflon</td>
</tr>
<tr>
<td>Transmissible mink encephalopathy (TME)</td>
<td>Mink</td>
</tr>
<tr>
<td>Chronic wasting disease (CWD)</td>
<td>Elk, mule-deer (in the USA)</td>
</tr>
<tr>
<td>Bovine spongiform encephalopathy (BSE)</td>
<td>Cattle, captive exotic ruminants</td>
</tr>
<tr>
<td>Feline spongiform encephalopathy (FSE)</td>
<td>Cats, captive exotic felids</td>
</tr>
<tr>
<td>Creutzfeld–Jakob disease (CJD)</td>
<td>Humans</td>
</tr>
<tr>
<td>Variant CJD (vCJD)</td>
<td>Humans</td>
</tr>
<tr>
<td>Gerstmann–Straussler–Scheinker syndrome (GSS)</td>
<td>Humans</td>
</tr>
<tr>
<td>Fatal familial insomnia</td>
<td>Humans</td>
</tr>
<tr>
<td>Kuru</td>
<td>Humans</td>
</tr>
</tbody>
</table>

### Table 2 The number of cases of indigenous BSE recorded in different countries each year between 1993 and 1999

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Britain</td>
<td>34370</td>
<td>23945</td>
<td>14302</td>
<td>8016</td>
<td>3179</td>
<td>2133</td>
<td></td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>459</td>
<td>345</td>
<td>173</td>
<td>74</td>
<td>18</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Denmarkb</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>18</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Irish Republic</td>
<td>16</td>
<td>18</td>
<td>15</td>
<td>73</td>
<td>83</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Liechtenstein</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Luxembourg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>3</td>
<td>12</td>
<td>14</td>
<td>29</td>
<td>106</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>29</td>
<td>64</td>
<td>68</td>
<td>45</td>
<td>14</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

*aExcluding Canada, Falkland Islands, Germany, Italy and Oman that have reported cases only in imported animals.

*bOne case confirmed in February 2000.

Further comments can be made regarding this issue. Studies on the UK cases of vCJD confirmed by July 2000 have, thus far, still failed to implicate anything other than BSE-contaminated foodstuff as the likely cause of their disease. This likelihood is reinforced by the knowledge that TDE agents are able to withstand the temperatures used to cook food by boiling, oven-roasting, pressure-cooking and microwave exposure. The potentially escalating incidence of vCJD has been of particular interest in European countries that appear to have failed, thus far, to have controlled their BSE epidemics (Table 2). It is also the subject of international scientific interest because it represents the first relatively definitive evidence that an animal TDE can transmit to humans. This contrasts with the longstanding experience that occupational or dietary exposure of humans to the agent that causes scrapie in sheep, goats and moufflon does not appear to result in the transmission of scrapie to humans, despite the endemic nature of scrapie in a number of countries.

---


2 The Nature of TDE Agents

TDE agents have been considered to be simply rogue forms of the host's normal prion protein (PrP) that resist catabolic destruction by proteolytic enzymes.\(^4\) This has been considered to result from post-translational structural changes in PrP that are induced by contact with already-modified, disease-specific forms of PrP.\(^5\) The progressive accumulation of the modified, amyloidogenic form of PrP eventually results in the formation of fatal pathological deposits in the central nervous system, within which there is the highest level of expression of PrP. However, the 'protein-only' hypothesis is not universally accepted because it fails to adequately explain the individual phenotypic characteristics of the many strains of TDE agents that can be propagated in mice with the same \textit{PrP} genotype, and the stability of the strain-specific phenotype of the BSE agent in mice regardless of its previous passage history through various mammalian species with different \textit{PrP} genotypes.\(^6\) Some therefore consider that, although it is generally agreed that a modified form of PrP is an essential component of the infectious agents, additional (possibly non-host) informational molecules such as nucleic acids are required to explain strain diversity, but none have yet been identified.\(^7\)-\(^10\)

3 Routes of Transmission

\textit{Genetic}

It is considered that some of the TDEs are caused through the inheritance of \textit{PrP} genotypes that results in the spontaneous production of a disease-specific form of PrP, or that spontaneous somatic mutations in the \textit{PrP} gene could have the same effect. The high incidence of scrapie in UK sheep with 'scrapie-susceptible' \textit{PrP} genotypes was considered by some to constitute evidence that it is a genetically transmitted disease, but identical genotypes were subsequently identified in countries where scrapie does not occur.\(^11\) This clearly demonstrated that susceptibility to scrapie, but not the disease itself, is inherited. Also, GSS is a familial human disease that is thought to be inherited as a direct result of the mutation of proline to leucine at codon 102 of the \textit{PrP} gene. However, when transgenic mice were created that exactly reproduced this same single amino acid change, they did not develop a spontaneous neurological disease. They were, however, much more susceptible to experimental challenge with a human-derived GSS agent, compared with other human-derived agents.\(^12\) These types of studies

\(^8\) B. Chesebro, \textit{Science}, 1998, 279, 42.
suggest that TDEs which appear to be inherited may result from the inheritance of disease susceptibility rather than the actual diseases.

**Iatrogenic**

The accidental transmission of TDEs has occurred through the use of contaminated medicinal products such as vaccines or hormones, or through the use of contaminated surgical instruments or medical devices.\(^\text{2,13}\) Such incidents have confirmed what had already been indicated by experimental studies, namely, that disease occurs most rapidly after the introduction of infectivity into the central nervous system. However, peripheral routes (e.g. intramuscular or subcutaneous) are also effective but result in longer incubation periods.

**Oral/Dietary**

With BSE, there is convincing evidence that the epidemic was fuelled by feeding cattle with ruminant-derived meat and bone meal (MBM)\(^\text{14}\) that remained infected after processing by the rendering procedures used in the UK during the 1980s.\(^\text{15}\) With scrapie in sheep, it is also widely considered that a highly significant factor in the transmission of this disease is the oral exposure of lambs to the infectivity that is known to be potentially present in the placentae or foetal membranes of infected mothers. Exposure to such infectivity is considered to arise post-natally through direct contact or via contaminated bedding, pasture, etc. The relevance of this route of infection has been demonstrated by the successful transmission of scrapie to sheep that were dosed orally with foetal membranes from scrapie-affected sheep.\(^\text{16}\)

The putative association between cannibalism and the expansion of the kuru epidemic in a tribal population of New Guinea was initially interpreted as definitive evidence that the consumption of kuru-infected brain-tissue was the reason for the expansion of this epidemic. Although there is little doubt that the tissues of kuru victims were removed in some form of reverential ritual, anthropological studies have revealed that visiting research workers never observed the actual consumption of the brains of kuru victims.\(^\text{17}\) It was later acknowledged that infection after contact with potentially infected tissues was 'most probably through cuts and abrasions of the skin or from nose-picking, eye rubbing or mucosal injury'.\(^\text{18}\)

TME occurs rarely and sporadically, and a foodborne source seems likely but remains unconfirmed. It was claimed that the most recent outbreak (in the USA) occurred in mink that had only been fed on bovine tissues obtained from ‘downer’

---


Mad Cows, Demented Humans and Food

cattle. This was considered to be an indication that cattle in the USA might be infected with a BSE-like disease that was transmissible to mink, but the active cattle surveillance programme in the USA has failed to reveal the presence of any BSE-like disease to date.

Until vCJD emerged, no studies had been conducted to determine the precise mechanisms whereby TDE infections might become established after dietary exposure. In this respect, experimental studies have now been carried out to determine (a) the route(s) by which infection reaches the central nervous system, and (b) which cellular components of the intestinal wall express PrP at sufficiently high levels to represent likely routes of entry for TDE agents. Following the oral challenge of hamsters with hamster-passaged scrapie agent, it has been demonstrated that the earliest appearance of the disease-specific form of PrP in the brain results from transmission through the vagal nerve; infectivity also appears to consistently, but more slowly, reach the brain by traversing through enteric lymphoid tissue, spleen, splanchnic nerves and the thoracic region of the spinal cord, from which it travels to the brain. The latter route has also been found to occur after peripheral infection with mouse-passaged scrapie, and these studies have confirmed the importance of agent replication in the follicular dendritic cells of the spleen before infectivity is delivered to the central nervous system. Studies on the distribution of normal PrP in the intestinal wall of humans have demonstrated its presence in nerve endings that form intimate associations with enteric epithelial cells, and show that only a thin layer of epithelium stands between ingested TDE agents and host PrP.

The Occurrence of BSE in Species other than Cattle through Ingestion of the Infectious Agent. Despite the uncertainties regarding the transmission of some other TDEs by a dietary route, there is convincing circumstantial evidence that BSE has transmitted to a variety of species, other than cattle, by this route. In the UK, 87 cases of feline spongiform encephalopathy (FSE) have been identified in domestic cats since 1990. FSE had not been observed previously and, except for one unexplained case in Norway, has only been observed in the UK. The only apparent route of infection was dietary, and strain typing has shown that the FSE agent is identical to that of BSE. Similarly, BSE has apparently transmitted to a variety of exotic felid and ruminant species housed in, or originating from, UK zoological collections (Table 3). In the case of the felid species, these are likely to have acquired their BSE-like disease through the feeding of putatively BSE-infected bovine tissues. In the case of the ruminant species, their disease probably resulted from feeding them with proprietary feedstuff that contained BSE-contaminated MBM prior to the 1988 ban on such a practice. Confirmatory evidence that these types of diseases were actually caused by the BSE agent has been formally

obtained, at least for kudu and nyala, by demonstrating that the strain of agent causing their diseases was identical to that of the BSE agent. It thus seems beyond reasonable doubt that all of the BSE-like diseases in exotic felid and ruminant species have been caused by the BSE agent through the ingestion of contaminated feed material.

The Association between vCJD and Dietary Exposure to the BSE Agent. Emotive headlines in the UK media during the mid-to-late 1980s suggested that there should be concern about the consumption of beef with regard to the transmission of BSE to humans. However, the product recognized traditionally as beef in the UK would be the muscle tissue of relatively young cattle that were unlikely to have been fed MBM. Even if such cattle were potentially infected, their usual age at slaughter would have meant that they were unlikely to be harbouring detectable levels of BSE infectivity in any of their tissues. In considering potential human health hazards, attention should have been focused on products (other than beef) obtained from older dairy cattle that were likely to have been fed MBM. Such animals were usually slaughtered at an age where they could be close to displaying clinical signs of BSE, and therefore have infectivity in their central nervous system.

As will be discussed later, there is overwhelming evidence that vCJD is caused by the BSE agent. Studies initiated in 1996 have still failed to implicate anything other than dietary exposure to the BSE agent as the cause, but its association with any particular type of food product has not been demonstrated. One problem is that, in common with other TDEs, vCJD is likely to have long and variable incubation periods that could extend to decades. The most significant period of exposure of the UK population to the BSE agent would have been in the 1980s when the BSE epidemic was expanding rapidly, until late 1989 when the first significant measures to protect human health were introduced. At the CJD Surveillance Unit in Edinburgh, the dietary habits of individuals who became vCJD victims have been compared with controls without demonstrating any apparent significant differences. Nevertheless, there are intellectual or recall-bias problems in interviewing cases, or their relatives, regarding the eating habits of such individuals up to 20 years ago. However, it was observed that mechanically recovered meat (MRM) that was likely to have been commonly incorporated into cheap food products had featured significantly in the diets of both the vCJD cases

### Table 3

<table>
<thead>
<tr>
<th>Felid species</th>
<th>Number affected</th>
<th>Ruminant species</th>
<th>Number affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ankole</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Bison</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Eland</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Gemsbok</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greater kudu</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nyala</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oryx</td>
<td></td>
</tr>
</tbody>
</table>

*Strain typing has demonstrated that the causal agents are identical to that of BSE.
Mad Cows, Demented Humans and Food

and the controls. It was recognized that MRM was likely to have contained particles of bovine spinal cord that could contain potentially high levels of the BSE agent, and its incorporation into human foodstuff was prohibited in Great Britain in 1994. Also, when the apparently upwardly spiralling increase in the incidence of vCJD was reported in July 2000, consultants from the CJD Surveillance Unit in Edinburgh were reported in the media to have again suggested that a significant factor might have been 'institutional cuisine' in the past whereby organizations such as works canteens and school meal services may have opted to use cheaper food products that were more likely to have contained MRM. There is thus the enigma that, although respected experts appear to believe that MRM has been a potentially significant dietary vehicle whereby BSE might have been transmitted to humans to cause vCJD, this is not reflected in the studies in which the dietary habits of cases were compared with controls. However, even by July 2000, the number of cases of vCJD was not high enough to exclude chance or random factors in its aetiology. With regard to the potential consumption of MRM, the distribution of BSE infectivity in any given batch was unlikely to have been homogeneous. Thus, in social groups consuming food containing infected MRM at the same meal, most might have received portions that contained either no BSE infectivity, or insufficient to establish vCJD infection. In contrast, another individual might have consumed a portion of the same food that contained sufficient infectivity to have infected a number of people if the BSE infectivity had been more evenly dispersed throughout the food product. The statistical situation may also have been clouded by the operation of other 'chance factors.' For example, vCJD has only occurred thus far in individuals that are homozygous for methionine at codon 129 of their \( PrP \) genes. However, experience with the accidental transmission of human TDEs to humans suggests that vCJD is also likely to occur in valine homozygotes and methionine/valine heterozygotes. The simple explanation as to why vCJD has not yet apparently occurred in such genotypes is that its incubation period may be longer in such individuals. A more worrying alternative is that the clinical and neurohistopathological features of vCJD in these genotypes might not be sufficiently distinctive to differentiate it from sporadic CJD. If this proved to be the case, evidence of its occurrence might only be revealed by the relatively long-term observation of an unexpected increase in the incidence of sporadic CJD.

There are also other factors, including genetic differences at loci other than that of the \( PrP \) gene, that might influence the likelihood of individuals succumbing to vCJD. Also, although vCJD is clearly a neurological disease, its pathogenesis appears to involve an important earlier stage of agent replication within the lymphoreticular system.\(^2^4\) Thus, if the immune system of individuals happened to be stimulated at the time of their exposure to the BSE agent, this might render them more susceptible to infection. In addition, in what appears to be a food-borne disease, lesions within the gastrointestinal tract at the time of exposure might enhance the likelihood of infection.

The Scale of Dietary Exposure of the UK Population to the BSE Agent. Because of the considerable variety of species, including humans, that have become infected with BSE, the causal agent has frequently been considered to be particularly promiscuous. In 1988 and 1989 the first significant control measures were introduced to minimize the exposure of ruminants to infected feed, and humans to infected food, respectively. However, before the end of 1989, food was likely to have contained significant amounts of BSE infectivity. By that time, although more than 11,000 cases of BSE had been confirmed in Great Britain, it has been calculated that 446,000 infected, but apparently normal, cattle would have been processed in abattoirs to produce food. Scrapie is endemic in the UK but its apparent incidence, and the quantity of sheep processed through abattoirs, is not high enough to have resulted in its contamination of the food supply with scrapie agent to anything like the extent that the BSE agent did, and no association has been found between sporadic CJD and dietary or occupational exposure to scrapie agent. However, it is interesting to speculate as to whether the apparent absolute species barrier between sheep and humans (or other species) would have been so resilient if the dietary level of scrapie agent had been as high as that for the BSE agent.

4 The Evidence that vCJD is Caused by the BSE Agent

The hypothesis proposed in 1996 that vCJD resulted from exposure to the BSE agent is now supported by experimental data. It had already been demonstrated that the patterns of incubation periods in a panel of inbred strains of mice were essentially the same when injected with brain tissue from (a) British or Swiss cattle with BSE, (b) domestic cats from the UK with FSE, (c) exotic ruminants with BSE-like diseases and (d) goats, pigs and sheep that had been experimentally infected with the BSE agent. These incubation period patterns were quite unlike those for any other TDE agent. Similarly, the regional distribution and severity of spongiform changes in the brains of each of the different strains of recipient mice were the same for all of the isolates tested, but were dissimilar to those associated with other TDE agents. These studies convincingly demonstrated that the BSE agent is a single strain that has (a) affected cattle in Switzerland and the UK, (b) caused FSE, (c) infected exotic ruminants and (d) retained its distinctive phenotype after experimental passage through goats, pigs and sheep. When the brain tissues from cases of vCJD were injected into the same panel of mouse strains, the same picture emerged. This was entirely different from the pattern resulting from challenge with the brain tissues from cases of sporadic CJD, and provided overwhelming evidence that the BSE agent is the cause of vCJD. Confirmatory evidence was provided by the demonstration that the glycosylation patterns of the disease-specific forms of PrP derived from cattle with BSE and


humans with vCJD were essentially the same, but different from those derived from animals or humans with other TDEs.  

5 Reasons for the Occurrence of BSE in the UK and Elsewhere in Europe

It remains an open question as to whether the BSE agent was already present as a covert bovine pathogen before the UK epidemic occurred, or whether it was caused by the survival of a single thermostable strain of scrapie agent from sheep that survived the rendering processes used to manufacture MBM that was fed to cattle and other species. It is clear that the rapid expansion of the UK epidemic of BSE was due to feeding cattle with MBM that had been manufactured by the rendering industry from the tissues of BSE-infected cattle discarded mainly by abattoirs.  

Based upon the clinical symptoms of BSE, there have been anecdotal reports that this disease has occurred in the past. However, such reports cannot be seriously considered because UK field veterinarians with considerable experience of BSE still misdiagnose around 20% of suspects on the basis of their clinical symptoms. Also, archival studies on the brain tissue of cattle failed to reveal any neurohistopathological evidence that BSE existed in the past.  

Indigenous BSE does not appear to have occurred in countries that unwittingly imported infected cattle from the UK and that later developed BSE. Thus, the epidemics that have occurred in other countries are more likely to have been precipitated through their importation of MBM from the UK, before this practice was prohibited. It is relevant that UK renderers exported more MBM into the international market in 1989 than ever before, even though the BSE risks must have been known to their customers because of the British ban on feeding ruminant-derived proteins to ruminants introduced in 1988. The international brokering system for MBM does not generally permit the identification of countries that imported UK-derived MBM during 1989 or in any other years. However, BSE has only occurred so far in European countries, and has been shown (when tested) to have been caused by the single strain of infectious agent found in Britain. This appears to constitute relatively convincing evidence that BSE became established in other European countries through their importation of UK-derived MBM before such trading was prohibited.

6 Measures Introduced to Enhance the Safety of Bovine-derived Food Products

Considering that no animal TDE had ever been shown to transmit to humans in the past, it is not surprising that the initial scientific opinion was generally that BSE was unlikely to transmit to humans but that this was not impossible. During the mid to late 1980s, the media frequently reported the opinions of self-appointed experts who proclaimed that BSE would decimate the human population, but they failed to present convincing scientific arguments to support this hypothesis.

The fact that BSE eventually proved to be transmissible to humans was therefore largely a matter of serendipity rather than a foregone conclusion based upon solid scientific reasoning. Before vCJD occurred, governmental agencies and politicians had been obliged to consider a worst-case scenario in which there might be a significant risk to humans from the BSE agent. Consequently, many regulations were introduced in the UK, the EU and elsewhere to protect animal and human health at that time. Additional regulations were introduced when it became clear that there was an association between BSE and vCJD. The current regulations are complex, and only a simple overview of the effects of the most significant control measures will be considered here.

In 1988, a ban on feeding ruminant protein to ruminants was introduced in Great Britain because epidemiological studies clearly implicated the feeding of MBM to cattle as the cause of the expansion of the BSE epidemic.\textsuperscript{14} Although cattle were usually initially fed MBM as calves, they did not generally display clinical signs of BSE until they reached an average age of five years. Thus, the benefit of the feed ban was not experienced until 1993, when a downward trend in the incidence of BSE was observed. Switzerland also introduced a ban on feeding meat and bone meal to ruminants in 1990. Even though BSE has not occurred in its cattle population, the USA also introduced a ban on feeding ruminant-derived proteins to ruminants. In 1994, EU regulations were introduced that required MBM to be manufactured using steam under pressure at 133 °C; this resulted from the early release of data produced from rendering studies involving BSE-spiked raw materials which showed that other rendering procedures were not reliable.\textsuperscript{15} In 1994, the EU declared that all types of mammalian proteins should be excluded from ruminant diets unless it could be reliably demonstrated that ruminant protein was being excluded. After the emergence of vCJD in 1996, this ban was expanded to include the feeding of animal proteins to any form of livestock in the UK.

In late 1989, the use of specified bovine offals (SBO) in foodstuff for human consumption was prohibited in Great Britain, and in 1990 this was extended to prohibit their use in ruminant or poultry feed. At that time, nothing was known about the distribution of BSE infectivity in the tissues of infected cattle; the decision as to what should be included in the SBO list was therefore based upon what was known about the distribution of infectivity in the tissues of sheep with scrapie, as judged by mouse bioassay. The bovine tissues that were consequently excluded under the SBO regulations were the brain, spinal cord, spleen, thymus, tonsils and intestinal tract (from the duodenum to the rectum) of animals that were more than six months old. These regulations were modified in 1991 to exclude SBO regardless of the age of the cattle. Milk obtained from suspect cases was also required to be destroyed.

Later studies involving mouse bioassays of the tissues of BSE-infected cattle demonstrated that the brain and spinal cord harboured significant amounts of infectivity, but that there was no detectable infectivity in the other SBO. Because infectivity is readily detectable in the lymphoreticular tissues of sheep with scrapie, the apparent absence of infectivity in such tissues derived from BSE-infected cattle was unexpected. However, it was recognized that these negative results could have arisen because of the species barrier between cattle
Mad Cows, Demented Humans and Food

and mice. Studies that are still in progress have shown that the efficiency of transmitting BSE to cattle by intracerebral challenge is around 500-fold greater than transmission to mice by the same route (Wells, personal communication). However, cattle injected intracerebrally more than seven years ago with pooled spleens and lymph nodes from BSE-infected cattle remain healthy (Wells, personal communication). This is strong evidence that the lymphoreticular tissues of cattle do not become infected with the BSE agent.

With regard to milk obtained from BSE-affected cows, bioassays have failed to detect any infectivity.29 Also, in studying the progeny of 126 beef suckler cows that developed BSE, no BSE has been observed in any of their 219 offspring that were estimated to have collectively consumed more than 111 000 litres of milk.30

As time progressed, a few non-SBO tissues from natural cases of BSE or experimentally infected cattle were shown to harbour infectivity. These included the retina, which resulted in the 1995 ruling that brain should be excluded from the SBO list and replaced by ‘the skull, including brains and eyes.’ Also, the finding of infectivity in the dorsal root ganglia that are adjacent to the spinal column led to the UK regulation introduced in 1996 (rescinded in 1999) that beef for human consumption would have to be deboned. Also in 1996, it was decreed within the EU that the export of beef and beef products from the UK (already restricted by regulations introduced in 1990 and 1994) should be completely banned. Although this decision was reversed in 1999, France and Germany were still prohibiting such imports in 2000, in contravention of EU law. Following the announcement in 1996 that vCJD was probably caused by the BSE agent, additional control measures were introduced in the UK to further protect human health. These included a selective cull of cattle considered to be most at risk of being infected, together with the offspring of BSE-infected cows. By the beginning of 2000, 76 000 at-risk cattle and 7376 offspring had been slaughtered. The OTMS (over thirty month scheme) was also introduced, whereby only cattle younger than 30 months can be used as a source of human food. By the end of 1999, 3.7 million cattle older than 30 months had been slaughtered under this scheme. Some of these carcases were sent for incineration but the remainder were rendered to produce more than 200 000 tonnes of tallow and 400 000 tonnes of meat and bone meal that have been stockpiled awaiting their safe destruction.

Within the EU, there had been concern that some member states were reluctant to accept the possibility that their cattle might be at risk of becoming infected with BSE, even when risk assessments suggested such a likelihood. Even when BSE eventually occurred in some of these states, there was a reluctance to introduce the full armoury of control measures that might be considered to be appropriate in view of the UK experience. Initially, it proved difficult to achieve any degree of agreement on the nature of legislation that might be applied throughout the EU to prevent the spread of BSE, and thus reduce any associated ruminant and human health hazards. However, agreement has now been reached which may have resulted from the fact that (a) within recent years, BSE has appeared in countries that previously appeared to be BSE-free, and (b) apart from the UK, where the incidence of BSE has been declining progressively over the

past seven years, other countries are not experiencing the same significant downward trend in its incidence (Table 2).

The new legislation that is to be applied throughout the EU as from October 2000 requires that animal tissues considered likely to represent a BSE risk (SRM: specified risk material) should be safely discarded. These are the skull (including the brains and eyes), tonsils, spinal cord and ileum of cattle over 12 months old. Also included are the skulls (including the brains and eyes), tonsils and spinal cord of sheep and goats over 12 months old. The spleens of sheep and goats are also required to be discarded, regardless of the age of the animals.

In view of the recognized higher BSE risk in Portugal and the UK (Table 2), the prohibited materials in these countries will be the entire head (excluding the tongue but including the brains, eyes, trigeminal ganglia and tonsils), the thymus, spleen, intestine and spinal cord of cattle over six months old. If cattle are more than 30 months old, the vertebral column (including the dorsal root ganglia) must also be discarded.

There is a legal requirement within the EU that animals must be stunned before they are slaughtered in abattoirs. Stunning is intended to render animals unconscious until death ensues through exsanguination, and can be achieved by (a) the passage of electrical current through the brain, (b) the use of a gun that discharges a captive bolt that percusses against the head of the animal but does not penetrate into the brain or (c) a similarly delivered bolt that penetrates into the brain.

Penetrative stunning is sometimes accompanied by pithing but this is subject to considerable geographical variation. The pithing process involves the introduction of a flexible rod into the hole created by penetrative stunning which is prodded and agitated into the the brain and cervical spinal cord. This minimizes the involuntary movement of the animals being slaughtered before their death through exsanguination, and reduces the risk of accidental injury to abattoir personnel.

It is known that severe brain trauma in humans can result in the appearance of microscopic particles of brain tissue in the bloodstream.\(^\text{31}\) When captive-bolts that inject air into the cranial cavity were used in cattle, relatively large particles of brain tissue were detectable in the jugular vein, heart or main pulmonary arteries of up to 33% of the cattle studied.\(^\text{32-34}\) However, these studies did not permit any conclusions to be drawn as to whether microscopic quantities of brain tissue might traverse through the lungs into the general arterial system and eventually lodge in tissues such as muscles that are used as food. An informal survey has concluded that the compressed-air captive-bolt system is probably little used, if at all, within the EU. Nevertheless, it has been recognized that the combination of penetrative stunning and pithing might release brain tissue into the bloodstream and permit BSE infectivity to sequester in tissues destined for


human consumption that would otherwise be free from infectivity. New EU legislation (operative from 31 December 2000) will prohibit the practice of pithing during the slaughter of cattle, sheep or goats intended for human consumption.

It has to be recognized that not all animal-derived constituents of human food products are obtained directly from carcasses processed in abattoirs. For example, blood is collected in abattoirs and then added to food products in an untreated or treated form. Also, products such tallow and gelatin are manufactured from tissues discarded by abattoirs, and can also be incorporated into food products.

Apart from the UK, blood collected from cattle that have been declared fit for human consumption is permitted to be included in human food products regardless of whether or not it is subjected to any further processing. Thus, untreated plasma can be found in sausages. Other blood products may be subjected to some form of heat processing before they end up in food products, but this is not required by any form of legislation. The relatively relaxed regulatory attitude to the incorporation of bovine blood into food products is largely based upon the knowledge that infectivity is generally undetectable in the blood of animals or humans affected by TDEs. However, the potential contamination of bovine blood with the BSE agent as a result of penetrative stunning and pithing in abattoirs has been recognized and addressed, as discussed above. Clearly, if there are opportunities in the abattoir for blood to become adventitiously contaminated with brain tissue or spinal cord material, this would introduce a significant doubt concerning the safety of food products that contained blood or blood-derived products. It is considered unlikely that this could occur in the licenced abattoirs from which blood is obtained for inclusion into human food. The EU continues to monitor this situation.

Information supplied by courtesy of the Gelatin Manufacturers of Europe (GME) shows that, worldwide, the gelatin industry annually converts over one million tonnes of raw materials from animals into 220000 tonnes of gelatin, of which 100 000 tonnes are produced in Europe. Asia, North America and South America are the next largest manufacturing areas, each with around 16% of the international market. Around 60% of the gelatin produced is used for food purposes, but much of that is produced from porcine skin. The highest use of gelatin in food products is in confectionery, followed by (on a decreasing scale) jellies, low fat and dairy products, and meat products. On a global scale, 25% of the gelatin used for food purposes is produced from bovine hides and bones. In Europe, this is somewhat lower at 15%, and not all of the bone material is sourced within Europe.

During the course of the BSE saga, the EU, FDA and other national authorities have maintained a watching brief to determine the nature of any potential problems associated with the use of gelatin in food or other products used by humans. Changing perceptions of risk have resulted in recommendations that raw materials should be obtained from areas with a low BSE risk, and that these should not contain bovine skulls or vertebral columns. Also, gelatin for human consumption is not permitted to be manufactured in the UK. Although the procedures used to manufacture gelatin are considered likely to result in a significant degree of inactivation or removal of the BSE agent, this has never been
D. M. Taylor

formally demonstrated. Consequently, the GME have funded a series of sophisticated validation studies to quantify this.

Although the manufacture of tallow for incorporation into foodstuff is prohibited in the UK, this practice is permitted elsewhere. It can be incorporated into a variety of products, providing that it has been manufactured from tissues declared fit for human consumption. However, this product appears to have an extremely low likelihood of being contaminated with the BSE agent, even when manufactured under worst-case conditions. Experimental rendering studies, in which abattoir waste was spiked with BSE-infected brain tissue at a level of 10%, produced tallow with no detectable infectivity, even though the most poorly inactivating rendering process produced MBM with an infectivity titre almost as high as that in the unprocessed raw materials.\textsuperscript{15}

7 The Question as to whether BSE could be Masquerading as Scrapie in Sheep or Goats

BSE can be transmitted orally to goats and sheep by feeding them as little as 0.5 g of brain tissue from BSE-affected cattle, and the ensuing disease is clinically and neurohistopathologically indistinguishable from scrapie.\textsuperscript{35} However, such studies are not necessarily relevant to the question as to whether goats or sheep might have become infected in the past by feeding them with MBM that was infected with the BSE agent. This is because the infectivity titre in MBM could not have been nearly as high as that in the BSE-infected brain tissue used in the oral transmission studies. It is difficult to resolve whether transmission of BSE to sheep or goats might have occurred in countries within which scrapie, but not BSE in cattle, is endemic, but where they might have been fed MBM imported from the UK. The situation is even more complicated in countries where both endemic BSE and scrapie occur if sheep or goats were fed MBM. In the UK, where such a situation exists, there are a number of ongoing studies designed to determine whether the BSE agent has infected sheep, but these may not produce unequivocal data. A further clue might be provided by strain typing some of the agents that have caused singleton cases of scrapie in 12 different Swiss sheep flocks during the 1990s. Such a study could be relevant because Switzerland appeared to be scrapie-free before the emergence of its BSE epidemic, and it is already known that the strain type of the BSE agent in Switzerland is the same as that of the British BSE agent. Although it would be considered unusual for only single sheep to become scrapie affected in all of these Swiss flocks, the occurrence of single, or few, cases of BSE in herds of cattle that were fed potentially BSE-infected MBM is not unusual. This is considered to have resulted from the uneven distribution of BSE infectivity throughout any given batch of MBM by virtue of the manufacturing process.\textsuperscript{36}

8 Future Trends

Since 1993, the year-to-year incidence of BSE in the UK has shown a significant

\textsuperscript{35} J.D. Foster, J. Hope and H. Fraser, Vet. Rec., 1993, 133, 339.

92
decline as a result of the introduction of progressively rigorous control measures, but it still remains to be seen whether or not complete eradication can be achieved. However, the same consistent downward trend has not been experienced in other European countries within which endemic BSE occurs (Table 2). It remains an open question as to what the eventual scale of the epidemics, and the commensurate risks to human health, will be in these countries. As has been discussed, the EU has introduced new measures to minimize the expansion of the incidence of BSE in countries already affected, and to avoid the spread of this disease to other countries. However, the UK experience has been that regulatory measures are only effective if they are adequately monitored. This is reflected in the current UK position whereby there are frequent visits by government inspectors to abattoirs, knackeries, hunt kennels, rendering plants, feed mills and incineration facilities to ensure compliance with all of the relevant regulations. There is also a strict policy that, where there is sufficient evidence, those that have broken the law will be prosecuted unless their transgressions were of an innocent nature. The UK has learned these lessons from bitter experience, but it remains to be seen whether or not other European countries accept the need, or are prepared, to commit such considerable resources to tackle their BSE problems.