Bovine spongiform encephalopathy and new variant Creutzfeldt-Jakob disease

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Bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob Disease (CJD) belong to a group of degenerative neurological disorders collectively known as the transmissible spongiform encephalopathies (TSEs). The group also includes scrapie of sheep and goats, kuru of humans, chronic wasting disease of mule deer and elk and transmissible encephalopathy of mink. These fatal diseases cause behavioural changes, alterations of sensation, changes in mental state and ataxia. The typical pathology is non-inflammatory vacuolation (spongiosis) in neuronal perikarya and in the grey matter neuropil. Occasionally, there may also be amyloid plaque deposition in certain regions of the brain and, less frequently, the spinal cord. All the diseases have long incubation periods which, depending on the host, may range from many months to several decades. Death is inevitable after a slow progressive illness. In this review, I shall cover the recent UK outbreak of BSE and its relationship to new variant Creutzfeldt-Jakob disease.

The nature of the causative agents of the TSEs remains a topic of scientific debate. The most favoured hypothesis, the prion hypothesis, is that the agents are composed of an altered form of a host-encoded protein known as PrP and lack a foreign nucleic acid. This hypothesis was originally proposed by Griffiths in the 1960s but has been promulgated and developed by Prusiner and colleagues. Multiplication of prions involves the conversion of the normal cellular form of the protein (PrP^c) to the abnormal prion form (PrP^sc). This conversion appears to be mediated by PrP^sc itself, probably through complexing of the two forms in endosome/lysosomal compartments in the cell. PrP^sc is relatively resistant to proteolytic digestion, accumulates in affected tissue and is strongly associated with pathological lesions. Transmission of the prion PrP^sc to uninfected animals synthesising PrP^c re-initiates the whole process. An alternative hypothesis, favoured by a number of prominent British researchers, is that an informational molecular component in addition to PrP^c, possibly a nucleic acid, is present in the infectious agent. This ‘virino’ hypothesis provides a more plausible explanation for the existence of multiple strains of TSE agents. Strains...
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are difficult to envisage under the prion hypothesis, but, as virinos, the strains might vary in their nucleic acid component. There is increasing evidence for the existence of strains, and 'strain typing' has become accepted as a major criterion for determining relatedness between different isolates (see below).

The BSE outbreak

In contrast to scrapie which has been endemic in UK sheep for at least 200 years, BSE was first diagnosed in UK cattle in November, 1986. This was the first observation of a spongiform encephalopathy in bovines. Heightened surveillance through 1987 suggested that an epidemic was underway and that the number of cases would continue to rise sharply. The epidemiology was unlike that of most infectious diseases, in that cases occurred randomly across the UK and there was an absence of distinct foci of high incidence infection. Several possible causes of the spread of this disease were investigated by Wilesmith and colleagues, including possible links with agricultural chemicals, veterinary products, contact with other animal species, imported semen, etc. Completion of a case study of 200 affected herds indicated that the most likely cause was the use of proprietary cattle feeds containing meat and bone meal prepared from abattoir waste. It was suggested that the meat and bone meal was contaminated by the sheep scrapie agent which had survived heat treatment and that this had transmitted to cattle. This hypothesis was given substance by the observation that there had been changes in the rendering industry in the late 1970s which had led to decreased processing times through a change from batch to continuous methods and the abandonment of organic solvent for fat extraction. Such changes would have given the scrapie agent a better chance of surviving the rendering process. Moreover, they would also allow the survival of the agent in cattle waste and, therefore, facilitate cattle–cattle transmission of BSE.

Based on Wilesmith's conclusions, the UK Government implemented a ban on the feeding of ruminant-derived proteins to ruminants, in July 1988. It was anticipated that if consumption of meat and bone meal were the only source of the BSE infection for cattle, this single measure would bring an end to the epidemic by stopping any new infections. In any event, however, because of the long incubation period, (approximately 5 years), the number of clinical cases would continued to grow for several years.

In fact, the BSE epidemic reached a peak of just over 1000 cases per week in January 1993 (Fig. 1). Since then, the incidence has shown a steady fall. By 31 January 1998, there had been 170 259 confirmed cases...
Fig. 1 BSE in Great Britain – confirmed cases by month of clinical onset.
affecting 60.4% of dairy herds and 16.9% of beef suckler herds. The disease continues to display a very low average within herd incidence of just under 3 cases per 100 adult cattle per year. Both sexes and all breeds appear to be equally susceptible. This observation, and others, suggest that there is no genetic predisposition to the development of BSE among cattle, although this cannot be ruled out. Of the total confirmed cases to January 1998, 35 111 were in animals born after the date of the feed ban (July 1988). This observation suggested that there are methods of transmission other than via meat and bone meal and/or that the feed ban had been incomplete. Recent studies suggest a low level of maternal transmission of BSE of approximately 10% of calves born in the last two years of incubation. However, this rate of maternal transmission is insufficient to account for the majority of the ‘born after the ban’ cases. The continuing rise of such cases into 1996 suggested that meat and bone meal had continued to find its way into cattle feeds long after July 1988. This was one of the factors that led to a total ban on the use of meat and bone meal for all farm animal feeds from August 1996.

**Pathogenesis**

Studies of scrapie in sheep and goats, notably by Hadlow and colleagues, have established the pathogenesis of disease following inoculation via peripheral routes. These experiments indicate that, in addition to CNS tissue, other organs such as spleen and lymph nodes may be positive for infectivity in the early stages of incubation. In an attempt to gather similar information for BSE in cattle, scientists from the UK Ministry of Agriculture, Fisheries and Foods (MAFF) have conducted pathogenesis experiments in which cattle were fed BSE infected material. Following infection, animals were then sacrificed at 4-monthly intervals. Numerous tissues, including those known to be involved in the pathogenesis of sheep scrapie, were then bio-assayed by mouse inoculation. The first tissue to show positive using this procedure was the distal ileum which showed positive at 6 months post inoculation. Thereafter, brain, spinal cord and dorsal root ganglia became positive after 32 months. The animals involved in this study received a high dose of BSE and many showed signs of clinical disease before termination of the experiment at 40 months.

**Risks to human health**

Early advice to MAFF was that BSE would be unlikely to pose a risk to human health since there was no evidence that any other animal...
encephalopathy could transmit to humans via the oral route. Moreover, it appeared that the most likely explanation for the origin of BSE was sheep scrapie \(^5,12\) and, therefore, it might well behave similarly in terms of its transmission potential. Evidence that scrapie does not pose a risk to human health is provided by epidemiological studies which indicate that the incidence of CJD is very similar in countries surveyed, whether or not sheep meat is eaten in any quantity and whether or not sheep in those countries have endemic scrapie. It is clear that, in a number of those countries, considerable amounts of scrapie infected sheep meat, including CNS tissue, is eaten.

In spite of this observation, however, concerns about the possibility of transmission of BSE to humans grew during the late 1980s. Evidence in the literature indicated that transmission of TSEs between species could not be readily predicted. Moreover, it became clear that BSE could transmit to another species (mice) via the oral route \(^13\). Scientists became concerned that BSE could have a different transmission potential from that recognised for sheep scrapie and, therefore, could pose a risk for other animal species, including humans, that may come into contact with infected bovine tissue. The long incubation period of TSEs made it likely that many pre-clinical BSE infected cattle would enter the human food chain. Such animals would be virtually impossible to identify, since even those in late stage incubation would only be detectable by histological examination at a level of detail which would far exceed the capacity available. This worry threatened the UK beef industry and led to the introduction of the specified bovine offals (SBO) ban in late November 1989 for England and Wales and January 1990 for Scotland and Northern Ireland. This prohibition order prevented the inclusion of brain, spinal cord, thymus, intestine, tonsil and spleen into the human food chain. Following the removal of SBO, the remainder of the carcass of all animals from UK abattoirs were deemed safe for human consumption. To watch for possible evidence of transmission to humans, however, the MAFF and Department of Health consultative committee on TSE research, recommended an improved structure for monitoring CJD incidence in the UK by the establishment of a national CJD surveillance centre. The remit of the centre was to monitor carefully all CJD occurring in the UK and look for signs of increased incidence, a change in the pattern of presentation or a change in the clinical and pathological manifestations of CJD indicative of the emergence of a new type.

**BSE transmission to other species**

In 1989, a new spongiform encephalopathy appeared in domestic cats in the UK \(^14\). This was the first description of a TSE in cats anywhere in the
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world. Questions were immediately raised as to whether this constituted BSE transferred across a species barrier to a novel species. This seemed a likely possibility given that cats were exposed to substantial quantities of contaminated bovine offal via pet foods. Transmissions to exotic species was also suspected when new TSEs of the greater kudu and the Arabian oryx appeared\textsuperscript{15}. Strain typing studies by Bruce and colleagues\textsuperscript{16} have since established that the agent causing these new TSEs was indeed the same as that causing BSE. The observations added to the growing concern that BSE was more promiscuous, in terms of its potential to transmit to other species, than known scrapie strains. Suspicion that BSE may have transmitted to humans was aroused in late 1994 and 1995, when CJD was diagnosed in several farmers and in unusually young individuals in the UK.

New variant Creutzfeldt-Jakob disease (nvCJD)

Events took a dramatic turn on 20 March 1996 when, after taking advice from its Spongiform Encephalopathies Advisory Committee (SEAC), the UK government announced that a new variant form of Creutzfeldt-Jakob Disease (nvCJD) had emerged, affecting 10 young people in the UK over the previous 14 months. SEAC concluded that the most likely cause of this apparently new human disease was exposure to BSE, probably through the consumption of infected bovine offal prior to late 1989, from when the inclusion of such material in human food was banned. This, announcement pre-empted a publication from Will, Ironside and their colleagues\textsuperscript{17} which described the nvCJD as being different from previously recognised sporadic (sp) CJD, by several criteria, as summarised in Table 1.

Available evidence suggested that nvCJD did not exist before 1994 and was not present in parts of the world unexposed to BSE. The conclusion, therefore, was that this was a new disease that had arisen in a time and geographical location that was entirely consistent with a link to BSE. Given the rarity of new TSEs generally, emergence of a new form of CJD in the UK at this time was unlikely to be a mere coincidence. The SEAC committee, therefore, took the view that BSE was the most likely cause. In other words, the dramatic and chilling conclusion was that BSE seemed to have transmitted to humans. Given the scale of the BSE epidemic in cattle and the extent of the consumption of carcasses from infected animals, the possibility arose that there might be a large scale epidemic of nvCJD.

Since March 1996, there have been a further 14 cases of nvCJD (\textit{i.e.} 24 in total up to the end of February 1998) which have appeared as a
Table 1 Characteristic features of nvCJD and differences from previously recognised sporadic (sp) CJD

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<th>Feature</th>
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<tr>
<td>An early age of onset or death (average 27.6 years compared to 64 years in spCJD)</td>
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<tr>
<td>A prolonged duration of illness (average 13.1 months, compared to 4 months for spCJD)</td>
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<td>A predominantly psychiatric presentation including anxiety, depression, withdrawal and behavioural change which progresses</td>
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<td>After a period of weeks or months the development of a cerebellar syndrome with gait and limb ataxia. Myoclonus develops in the majority of patients and in some is preceded by choreiform movements</td>
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<td>Forgetfulness and memory disturbance develop, often late in the clinical course, but progress with the development of severe cognitive impairment and a state of akinetic mutism in the majority of cases</td>
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<td>The typical EEG appearances of CJD are absent</td>
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<td>The most striking and consistent neuropathological feature is amyloid plaque formation extensively distributed throughout the cerebrum and cerebellum</td>
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steady trickle. At this stage, there is no sign of an upturn in rate of presentation that would portend a large epidemic. Some have, therefore, raised the question of whether the disease is indeed linked to BSE or whether there is some alternative cause. However, further strong scientific evidence has accumulated that confirms a link between the two diseases and no evidence of any kind supporting an alternative hypothesis has emerged.

The further evidence supporting a link is as follows. In mid-1996, Dormont and colleagues reported that in rhesus macaques, BSE produces a neuropathology that closely resembles the unusual form seen in nvCJD (especially the production of florid plaques and prominent involvement of the cerebellum). At the time of publication, it was difficult to assess the weight of this evidence, since in cattle and other nonprimate species, BSE does not cause this type of pathology. Nevertheless, the message was consistent with a connection – BSE causes the unusual vCJD type of pathology in a primate. It seemed likely, therefore, that this unusual pathology in humans was also caused by BSE.

The first molecular evidence of a link was provided by John Collinge and his colleagues who showed that the ratio of di-, mono- and unglycosylated forms of the PrP protein (the ‘glycoform profile’), extracted from the brains of victims, was a measurable characteristic of disease. In analysing a number of spCJD cases, they identified 3 distinct profiles (types 1–3). All cases of nvCJD examined, on the other hand, produced a different and yet consistent pattern (type 4), providing further evidence that this was a distinct disease. Moreover, the same type 4 pattern was observed in PrP taken from the brains of cattle with BSE,
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cats and kudu, established by Bruce and colleagues to have been naturally infected by the BSE agent, and a macaque experimentally infected with BSE. This was the first molecular evidence of a link between the two diseases. Again, at the time of publication, although indicative of a link, the weight of this evidence was also difficult to assess because of a lack of background information on whether the type 4 profile is common among other TSE agents, including those of sheep.

Recently, there have been two further publications that, taken together with those above, have surely put the link beyond reasonable doubt. Both reinforce the conclusion that nvCJD is indeed distinct from other, sporadic forms of CJD and provide a very convincing case that nvCJD is caused by the same ‘strain’ of agent that has caused the BSE epidemic.

There is a large body of evidence for the existence of multiple ‘strains’ of TSE agents. Strains were originally identified by Dickinson and colleagues, who noticed that different isolates will give reproducible incubation times and pathology in certain lines of inbred mice. To characterise strains, four or five inbred lines of mouse are used, including those homozygotes for prolonged scrapie incubation periods (p7p7), for short incubation period (s7s7,) and heterozygotes (p7s7). The strains differ in their incubation times in a particular mouse line and in the temporal sequence in which the different lines develop disease. Pathology is measured by semi-quantitatively scoring spongiform change in nine regions of the brain and expressing the results graphically as a ‘lesion profile’. This profile is reproducible and characteristic of a given strain. Previously, eight cases of BSE taken from different UK locations and different periods in the epidemic had been characterised by these methods. All displayed highly similar strain characteristics (incubation time and lesion profile) distinct from those observed in TSE strains isolated from other animals over many years. Importantly, strain characteristics can be maintained upon inter-species transmission as shown by BSE transmission to a pig, a goat and two sheep. Previously, observations of the BSE profile in 3 cases of feline spongiform encephalopathy, and in cases in kudu and nyala in British zoos, had been taken as evidence that BSE has infected these species.

The same type of analysis was applied to three nvCJD cases by inoculating brain material into mice. The results showed convincingly that the strain of agent from nvCJD is: (i) the same in each of the three cases studied; (ii) different from that of other forms of CJD; and (iii) indistinguishable from that of BSE. The mean incubation period of nvCJD in the RIII line of mice used was relatively short, which is characteristic of BSE whatever its source. Moreover, the lesion profile produced in these mice was almost superimposable on that produced by BSE from cattle, or from any other species accidentally or experimentally infected with BSE. The inescapable conclusion was that
nvCJD is indeed caused by the same agent as causes BSE. In the same study, CJD that has occurred recently in farmers was analysed. This behaved like sporadic CJD.

The final piece of evidence favouring a link was the further work of Collinge and colleagues. They carried out a large number of transmissions of CJD to both transgenic mice expressing the human PrP gene (Prn-p) and their non-transgenic counterparts. These studies indicate again that the agent of nvCJD is distinct from those of both sporadic and iatrogenic CJD. Moreover, nvCJD and BSE were highly similar: glycoform profiles (both ratios and band sizes) were indistinguishable, both shared an unusual clinical presentation (some of the nvCJD mice walk backwards) and although details of pathology are yet to be published in detail, the authors refer to ‘striking similarities’ in PrP deposition patterns.

Taken together, the observations on the pathology, epidemiology, and molecular analysis of nvCJD and BSE, provide compelling evidence that the two diseases are directly related to each other. Indeed, the consistency of the similarities makes any other interpretation highly implausible. This conclusion, however, does not necessarily indicate that the victims of nvCJD became infected by eating contaminated beef products, although this seems the most likely explanation. Other routes of transmission could include bovine derived medicinal products prepared before the late 1980s from when such materials were generally sourced from outside Europe. Figure 2 indicates the possible relationships between the two diseases that would be consistent with them being caused by the same strain of agent and appearing in the same time frame and geographical region. Figure 2A is the direct route from bovine products as discussed above; Figure 2B indicates that nvCJD victims could have been infected from the same source of agent that initiated the BSE epidemic but perhaps not directly from bovine material. The favoured explanation for the origin of BSE is that it came from sheep scrapie, which for reasons discussed above makes this relationship unlikely. However, a scrapie origin for BSE is far from proven. A third possibility, Figure 2C, is that there may be an intermediate host between cattle and humans: domestic cats, exotic ungulates, or even sheep? Formally it is not possible to distinguish between these models on present evidence. However, the timing of the appearance of the two diseases is most consistent with the model shown in Figure 2A.

So what is the likelihood of an epidemic on nvCJD? As discussed above, there has been no upturn, so far, in the rate of presentation of new cases. However, it is still too early to conclude that the rate of presentation will stay flat. Intuitively, if Figure 2A depicts the relationship between the two diseases, it would be surprising if the rate of presentation
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did not in some way reflect the dynamics of the BSE epidemic. The exposure of the UK public to contaminated bovine products must have been orders of magnitude higher in 1989 (and possibly later if the SBO ban was incompletely applied), than it was, in say 1985. As pointed out by Cousens et al, much depends on the average incubation time of nvCJD: the longer the time the higher the final figure is likely to be. At present, there are no data which allows us to calculate the average incubation time of BSE in humans. Nor is it possible to estimate the amount of infectivity (in terms of cattle infectious doses or mouse infectious doses) required to infect humans via the oral or other routes. The range of possible numbers of cases of nvCJD in the UK population in the years ahead, therefore, remains wide. One interesting observation to date is that all the nvCJD cases so far examined are homozygous for a common polymorphism in the human PrP protein, namely methionine at position 129. This raises the possibility that those homozygous for valine at this position and those heterozygous, (representing approximately 11% and 51% of the UK population, respectively) may be relatively resistant to infection and/or may require longer incubation times, and/or may have different symptoms, signs and pathological changes from the cases of nvCJD so far observed. Time will tell.

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