Variation on a theme of Creutzfeldt–Jakob disease: implications of new cases with a young age at onset

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Introduction

Creutzfeldt–Jakob disease (CJD) belongs to a group of human and animal diseases, distinguished from each other by differences in their clinical and neuropathological presentation or aetiology, but known collectively as the prion diseases. Prion diseases are unique amongst transmissible diseases in that the transmissible ‘agent’ does not appear to contain any nucleic acid and the only protein known to be associated with infectivity is host-coded. Prion disease is endemic in certain species, notably humans and sheep. It has also appeared in epidemic form in humans: for example, amongst tribal peoples of Papua New Guinea, where the disease is known as kuru, and in Western societies as a consequence of iatrogenic misadventure. Epidemic prion disease also occurs in animals: for example, in cattle (as the current epidemic of BSE (bovine spongiform encephalopathy) in Britain (Wells & Wilesmith, 1995)) and in farmed mink (Marsh, 1992). The BSE epidemic, which has been attributed to contamination of feedstuffs, also extends to several species of zoo animal, including kudu, nyala and large cats, as well as domestic cats. Age at onset has been an important clue to understanding the aetiology of both endemic and epidemic prion disease in humans and was particularly important in identifying those cases which resulted from treatment with human pituitary-derived growth hormone. The recent referrals of 10 cases of prion disease in Britain with an unusual clinical and neuropathological presentation and a young age at onset (now termed ‘variant CJD’ or vCJD (Will et al., 1996)) alerted the CJD Surveillance Unit in Edinburgh to the possible occurrence of a new disease. After considerable deliberation this led to the statement by the Health Secretary, to the House of Commons in the third week of March 1996, that the most likely explanation at present is that ‘these cases are linked to BSE before the introduction of the SBO (Specified Bovine Offals) Ban of 1989’. But this suspected link between BSE and vCJD is not proven and the arguments for, and against, this conclusion are subtle. The implications of these arguments, in terms of predicting the occurrence of further cases and in taking appropriate action, are wide ranging. This paper attempts to explain aspects of prion disease which are pertinent to these arguments.

The nature of prion disease

In order to assess the likelihood that vCJD is BSE-related one needs to know a little about the unusual nature of prion disease. Since the disease is not caused by a virus, bacterium or other known micro-organism, our knowledge of diseases caused by such agents may be misleading. For example, the ‘agent’ of prion disease is not inactivated by most conventional methods of disinfection and there is no evidence that prion diseases are airborne infections or simple contagious diseases. The term prion disease is used because of the essential involvement of an abnormal form of a host-coded protein (prion protein or PrP) in pathogenesis in all cases. The central involvement of PrP is proven because genetically modified mice, which do not express prion protein, are totally resistant to prion disease (Büeler et al., 1993). The prion protein gene, which is highly conserved across all mammalian species, encodes a protein of approximately 254 amino acids. It is a transmembrane protein which is expressed in neurones although its normal function is unknown. In humans there is a common polymorphism at codon 129 with allele frequencies (in Caucasians) of ~ 0·32 valine, ~ 0·68 methionine (Owen et al., 1990). It is widely accepted that the primary pathogenic event in prion disease is the conversion of the normal host-coded prion protein (PrPc) to an abnormal form (PrPse) which is thought to differ from PrPc only in its conformation (Pan et al., 1993). PrPc turns over rapidly in normal cell function, while PrPse is, at most, only partially broken down and therefore accumulates in tissue. Recent research suggests that it is specifically the interaction between PrPse and PrPc which induces the pathological events which underlie neurodegeneration and neural malfunction and which lead inevitably to death (Brown et al., 1996; Brandner et al., 1996). In brain, PrPse is associated with intraneuronal vacuolation which produces the definitive microscopical appearance of spongiform encephalopathy. In a proportion of cases, PrPse aggregates and is deposited as well-formed PrP-amyloid plaques or as diffuse PrP deposits which can be visualized by immunohistochemical methods. The probability with which PrPc converts to PrPse is dependent on its primary structure but the primary structure of the protein is not altered during disease. When PrPse gains entry to another animal (or human) it induces the conversion of the new host’s normal PrPc to PrPse leading to disease. An important recent finding has been that
PrP<sup>Sc</sup> can convert PrP<sup>C</sup> to PrP<sup>Sc</sup> in an *in vitro* cell-free system (Kocisko *et al.*, 1994) suggesting that this interaction is purely biochemical and does not involve an independent replicating agent. Further work now suggests that another host-coded protein, currently referred to as 'protein X', may be an essential accomplice in the interaction between PrP<sup>Sc</sup> and PrP<sup>C</sup> (Telling *et al.*, 1995). Many researchers now regard PrP<sup>Sc</sup> as the only constituent of the transmissible agent in prion diseases.

**The aetiology of prion disease**

A common misconception expressed by scientists and laymen alike is that 'every case of CJD must have been caught from somewhere'. It is part of the extraordinary nature of prion diseases that there is more than one aetiology. In any individual case, the disease is either *acquired*, *sporadic* or *genetic* in origin. A tendency to combine aspects of more than one aetiology has led to a great deal of misunderstanding. For example, the fact that prion disease can be acquired under certain circumstances, together with the occurrence of disease in parent and offspring under different circumstances, has led to a hypothesis of maternal transmission of the agent whereas a familial pattern of the disease is actually more consistent with a genetic interpretation (Ridley & Baker, 1995). The Spongiform Encephalopathy Advisory Committee (SEAC) recently reported that the increased incidence of BSE in the offspring of cows who develop BSE, compared with the incidence in the offspring of cows who did not develop BSE, was evidence of maternal transmission, although the data are also consistent with differences in genetic susceptibility. Again, if it were assumed that CJD is *acquired* from sheep scrapie one might conclude that the risk of transmission from animals to humans is low because endemic CJD is extremely rare. However, it is the *sporadic* and *genetic* cases which result in low levels of endemic disease; *acquired* cases of prion disease tend to occur in clusters or epidemics which may be extremely large.

(i) **Acquired human prion disease**

Acquired disease occurs when a person or animal (the host) is contaminated with infected material from another affected case (the donor). When the conditions leading to this contamination are achieved the result is usually an epidemic which may be small in numbers (though tragic when it involves humans) or massive, as in the case of BSE which has affected more than 160,000 cattle in Britain. These acquired cases are naturally occurring examples of the phenomenon of transmissibility which has been adequately demonstrated under experimental conditions. Transmissibility is facilitated when (i) there is no species barrier between host and donor (i.e. they are of the same species), (ii) the infecting material is deposited directly into the brain of the host, and (iii) the infecting tissue is brain, spinal cord or related tissue. The first two of these factors do not apply to the risk of transmission of BSE to humans. The Specified Bovine Offals (SBO) Ban 1989 prohibited the inclusion of bovine brain, spinal cord and many offals in human food and should, therefore, have protected the public from the third risk factor. But this measure could not protect the public from contamination occurring from the earlier part the BSE epidemic, which began in 1986, and it may not have been fully effective after it was first put in place.

The earliest known iatrogenic cases of CJD (numbering ~ six) reflect the three major risk factors described above, in that they involved brain contamination during neurosurgery using instruments previously used in other patients who died of CJD (Brown, 1996). The incubation period was < 2 years. Subsequently, ~ 25 cases of CJD have resulted from the use of pituitary (dura mater) tissue mainly during neurosurgery (Brown, 1996). The incubation period in these cases was from 1.5–12 years. About 75 cases have occurred as a result of the use of human pituitary-derived growth hormone (GH), given by intramuscular injection over the course of several years, to promote growth in people of short stature (Brown, 1996). It is supposed that a very small number of the pituitaries came from donors who had died with CJD. In different countries, which had slightly different protocols for the production of the hormone, the estimated incubation period varies with the proportion of people affected. Thus ~ 2.5% of growth hormone recipients in France have so far become affected, with a mean incubation of 8 years, ~ 1% of British recipients have developed illness, with a mean incubation of 12 years, and ~ 0.2% of American recipients have become affected, with a mean incubation of 18 years. This suggests that incubation periods are related to 'dose' of infectivity. The difference in incubation period between countries would be compatible with each manufacturing method producing a different, but *uniform*, degree of contamination throughout the growth hormone product. The longer incubation period in GH-CJD compared to the other iatrogenic cases probably reflects the peripheral rather than central route of infection.

Kuru claimed the lives of many hundreds of people in Papua New Guinea during the middle part of this century, probably as a result of cannibalism of affected relatives (Alpers, 1987). The higher incidence in women and their children is thought to be because the women prepared the bodies and ate mainly the internal organs including brain, which they also fed to their children, while the adult men ate mainly the muscle. But while there is plenty of anthropological data on the number, ages and kinship of affected cases, information about the precise details of cannibalistic practices is somewhat impressionistic (Glass & Lindenbaum, 1992). Judging from the age at onset of the youngest cases and the most recent, now elderly, cases, the incubation period is thought to range from 3–30 years ( Klitzman *et al.*, 1984). This may reflect different levels of...
contamination in different groups of people. If BSE were to cause CJD then it must be accepted that cases could occur for many years to come irrespective of whether a small or a large number of people become ill. The story of kuru suggests that handling and eating infected human brain is a possible route of transmission of disease but this neither proves, nor disproves, that eating brain or other organs from infected animals provides the same level of risk. If it is supposed that the cases of vCJD are BSE-related then they probably became infected near the beginning of the BSE epidemic, before the SBO Ban of 1989. The incubation period would therefore have been ~ 8 years. It is to be hoped that any contamination of human food has been very non-uniform such that this incubation period reflects unusually high levels of contamination in individual food items.

In GH-CJD, kuru and vCJD, ataxia may be a presenting or early neurological symptom whereas sporadic CJD (see below) usually presents as cognitive deterioration with ataxia becoming apparent shortly afterwards. This latter presentation is also seen in those acquired cases resulting from direct contamination of the brain. These differences have been used to support the view that vCJD (like GH-CJD and kuru) is acquired via a peripheral route. This argument has some merit, but it is not definitive, since a substantial proportion of sporadic cases of CJD (Brown et al., 1994) and most cases of the familial prion disease, Gerstmann–Sträussler–Scheinker disease, also present with ataxia.

(ii) Sporadic human prion disease

Sporadic cases of CJD, which at present account for > 80% of human prion disease (Masters et al., 1978), occur, by definition, without any known association with environmental factors or with other cases in relatives or acquaintances. The worldwide annual incidence of CJD is remarkably constant at 0.5–1 per million of the population (Brown et al., 1987). The incidence is higher in more highly developed countries, which probably reflects greater surveillance, since the incidence seems to rise with greater medical awareness (Will, 1996). It is notable that the incidence of CJD in Australia and Japan is consistent with the general worldwide incidence, despite scrapie (the prion disease of sheep) being virtually non-existent in these countries (Hourigan et al., 1979). Furthermore, cases of CJD have been recorded in India (Masters et al., 1978) where the population is largely vegetarian. This suggests that sporadic CJD is not related to exposure to sheep or other animal material in the human diet.

(iii) Genetic human prion disease

Some cases of human prion disease are associated with mutations in the PrP gene and occur in an autosomal dominant pattern within families (Baker & Ridley, 1992). Now that it has become possible to identify these cases by molecular genetics, it is clear that the phenotype of human prion disease is more variable than was originally perceived. The terms Gerstmann–Sträussler–Scheinker disease, Fatal Familial Insomnia and Atypical Prion Disease are used to describe some of these different clinical and neuropathological presentations. It is also clear, however, that misdiagnosis does occur such that a proportion of cases must be missed. While historical diagnoses may have been non-specific, e.g. cerebral atrophy, a diagnosis of Alzheimer’s, Huntington’s or Pick’s disease is still common (Ridley, 1994). Furthermore, as with most genetic diseases with onset in later middle-age, a substantial proportion of patients are unaware of a family history. This may be due to family breakdown, secrecy about illness or illegitimacy, or, with respect to death in a parent, early death from unrelated causes, death early in the course of disease but prior to diagnosis (e.g. suicide, accident related to ataxia or mental disability), misdiagnosis or, when age at onset is very late, failure to appreciate that rapid mental deterioration is something more ‘to be expected at their age’. A calculation of the number of ‘missed’ genetic cases can be made by investigating the supposed cause of death in obligate carriers of the mutation. It may be supposed from this that a number of sporadic cases of CJD will also be missed but these cannot be identified by this method. The impression is gained that the phenotype of sporadic CJD is more circumscribed than the phenotype of genetic prion disease. This is probably true, but it may also be true that atypical cases of sporadic prion disease are systematically misdiagnosed. This raises the possibility that cases of vCJD might also be missed.

The new variant of CJD (vCJD)

The new variant, vCJD, is described by Will et al. (1996). Ten cases have been identified in Britain, with onset since February 1994 and by the time of that publication (April 1996), eight of these patients had died. Identified deaths from CJD in Britain have risen steadily from 1970–1994, mainly as a result of greater ascertainment in the elderly (Will, 1996), but the addition of ~ five further cases per year is not remarkable given the annual variation. What is extremely unusual (but not unique) is the very young age at onset in these cases. The age at death ranged from 19–41 years (median 29). This compares with a mean age at death of 58 years (range 19–78) in 112 cases of sporadic CJD from a worldwide survey (Masters et al., 1981b) and a mean age at death of 65 years for 185 cases of sporadic CJD in Britain since 1990 (Will et al., 1996). Worldwide, death from sporadic CJD has previously been reported in fewer than 20 people aged under 30 years (Will et al., 1996).

The clinical presentation of vCJD differs subtly but importantly from sporadic CJD. The majority of vCJD patients were initially referred to a psychiatrist rather than a neurologist reflecting the prodromal behavioural changes seen in these patients. Although cases of sporadic CJD may be more likely
to be referred directly to a neurologist, their relatives usually report a prodromal stage of diagnostic uncertainty (Cathala & Baron, 1987). Early neurological symptoms in vCJD included ataxia, with dementia and amnesia developing later. Myoclonus, which is an important diagnostic indicator for CJD, developed late in the disease in only seven of the patients, and none of the patients showed the EEG pattern which is usually associated with CJD. In these respects vCJD does bear a somewhat greater resemblance to GH-CJD and kuru than to sporadic CJD although, as we have said, this similarity does not prove that vCJD is an acquired disease.

vCJD also differs substantially from sporadic CJD in its neuropathological picture. In vCJD this comprises mild spongiform encephalopathy in the cortex and moderate spongiform encephalopathy in the basal ganglia and cerebellum accompanied by neuronal loss and astrocytosis. More striking, and very consistent between cases, is the occurrence of numerous PrP-plaques throughout the cerebrum and cerebellum. These plaques resemble kuru-plaques but, additionally, are frequently surrounded by a zone of spongiform vacuolation. This particular type of ‘florid’ plaque in humans can be considered pathognomonic for vCJD but its aetiological significance is unclear. PrP-plaques are seen in ~25% of cases of CJD and ~75% of cases of kuru, diffuse PrP-deposits and large multicentric PrP-plaques are seen in Gerstmann–Sträussler–Scheinker disease (Masters et al., 1981a), and plaques may be seen in the cerebellum and spinal cord of patients with GH-CJD (Ironside, 1996) but none of these have quite the characteristic appearance of the ‘florid’ plaques of vCJD. Duration of illness is longer in cases of CJD and kuru with plaques compared to cases without plaques (Masters et al., 1981a) and duration of illness can be very extended in familial forms of disease in which plaque formation is common. vCJD also has a somewhat extended course of illness compared to sporadic CJD (Will et al., 1996). An interesting quirk in this story is that sporadic cases of CJD with plaques carry at least one valine at codon 129 of the PrP gene (Ironside, 1996) whereas all the cases of vCJD so far, for whom PrP-genotyping is available, have been homoygous for methionine at codon 129 (Will et al., 1996). Whether the difference in the incidence of plaques in CJD and kuru is related to the frequency of valine-129 in the population of Papua New Guinea or whether it reflects a difference between sporadic and acquired disease is not established. Similarly, the relationship between plaques and genotype in GH-CJD is not yet clear.

Although the occurrence of ‘florid’ plaques is currently definitive for vCJD in humans, it is not indicative of disease derived from BSE. Such ‘florid’ plaques are not seen in cattle with BSE, sheep, goats, pigs and mice to which BSE has been transmitted experimentally, or the cats and exotic ungulates which have become naturally infected as part of the current epidemic. Plaques with various types of morphology, none of which is quite identical to that seen in vCJD, are seen in some cases of sheep scrapie (Fraser, 1979), some experimental mouse/agent combinations (Bruce, 1981), some human cases (Masters et al., 1981a) and in prion disease of mule deer (Bahnmanyar et al., 1985). However, it has recently been shown that three cynomolgus monkeys injected intracerebrally with brain tissue from a BSE affected cow developed spongiform encephalopathy with ‘florid’ plaques clearly resembling those seen in vCJD whereas two monkeys injected with tissue from a case of sporadic CJD developed spongiform encephalopathy without plaques (Lasmézas et al., 1996). Histological sections from marmosets, which developed spongiform encephalopathy following injection with material from cases of BSE, scrapie, Gerstmann–Sträussler–Scheinker disease or CJD (Baker et al., 1993, 1985a) in experiments completed before the appearance of vCJD, are currently under review. Specifically, the similarity between the PrP deposits in the brains of these animals and the ‘florid’ plaques seen in vCJD is being assessed.

A case of vCJD in France

In addition to the 10 cases of vCJD reported in Britain, another case has recently been described in France (Chazot et al., 1996). This patient is important because he had never visited Britain. If his disease was acquired it could have been in a number of ways. It could, for example, have resulted from eating British beef products exported to France. If this is the case then it is not possible to say yet whether this indicates a widespread level of contamination of British food, sufficient to produce a case amongst those eating the relatively small proportion of that food exported to France, or whether it is just a statistical quirk that a British-food-related case should occur in France. Or his disease could have resulted from eating food made from BSE-affected French cattle. If this is so then it is possible that one case could occur from a very low level of BSE in France. The recorded number of cases of BSE in France is currently <20 (Chazot et al., 1996). However, there are reasons for doubting whether ascertainment of BSE in France is comprehensive. This is a surprisingly low figure given the number of calves exported from Britain to France during the 1980s and the number of tonnes of meat and bone meal, presumably contaminated with BSE material, legally exported to France (Butler, 1996). Furthermore, some cases of suspected BSE in Britain are found, on pathological examination, to have another neurological disease (Wells et al., 1995). Any shortfall in the number of ‘non-BSE’ neurological cases in France will be an indication of the number of cattle with symptoms compatible with BSE which are not being detected. This would suggest that some ‘real BSE’ cases in France are being missed. However, the appearance of vCJD in France could suggest that vCJD is not caused by eating BSE-affected material at all. If this is so then it must be expected that, with increased awareness, further cases of vCJD in parts of the world far removed from the BSE epidemic will be found. There can, however, be few places with adequate surveillance of CJD to which British bovine products have not been exported.
Could cases of vCJD have previously been missed?

This is an important question since, at present, the supposition that vCJD is related to BSE is based solely on the fact that cases of this type have never occurred before. The neuropathological picture in vCJD is striking and it is most unlikely that it would be missed if brain preparations were subjected to full PrP immunohistochemical analysis. But such an analysis depends on the disease being recognized as organic rather than 'functional' (vCJD may first present as a psychiatric disorder), a diagnosis of prion disease being suspected, and permission for a post mortem being given by relatives. Even an analysis depends on the disease being recognized as organic rather than 'functional' (vCJD may first present as a psychiatric disorder), a diagnosis of prion disease being suspected, and permission for a post mortem being given by relatives. Even amongst cases classified as 'suspected CJD' by the CJD Surveillance Unit, the post-mortem rate is only \(~ 70\%\) (Will et al., 1996). Until recently, the likelihood of a diagnosis of (ordinary) CJD being suspected in life for these patients would have been reduced by the insidious onset of behavioural and psychiatric symptoms, the young age at onset and the absence of an electroencephalogram (EEG) characteristic of CJD. Indeed, when first assessed, none of the 10 cases achieved a diagnosis of 'probable' or even 'possible' CJD according to the CJD Surveillance Unit criteria. However, once it has been recognized that a case which does not conform to the established criteria is, in fact, a case of CJD, then the chances of other cases with the same presentation being detected are increased. If cases of vCJD did occur between 1990 (when the CJD Surveillance Unit was set up) and 1994 and were missed, the same sort of cases occurring after that time might have been detected, even if surveillance differed between these times only in the influence of the first detected case on subsequent diagnostic decision making. The weakest link in producing adequate surveillance is in ensuring that health professionals across the country are aware of precisely what to look for. It is to the credit of the CJD Surveillance Unit and the other medical professionals involved, as many as five cases of vCJD per year could have been missed between 1990 and 1994.

The spongiform encephalopathy which is pathognomic of CJD is intraneuronal but may be obscured by or confused with grey matter vacuolation which accompanies rapid neurodegeneration, i.e. status spongiosus. A variable degree of vacuolation of the neuropil is seen in frontal-lobe dementia, a proportion of cases of Alzheimer's disease and other neurodegenerative diseases, and in a few childhood diseases, e.g. Alper's syndrome. In the past at least three patients aged < 30 years have been diagnosed as suffering from subacute sclerosing panencephalitis (SSPE) only to be re-diagnosed as CJD following reappraisal of the neuropathology (Kulczycki et al., 1991). We are not suggesting that neuropathologists are easily mistaken, but would point out that what may appear to be clear-cut when written up in a readily digestible form may be much more uncertain when first encountered. As an indication as to how difficult it is to perceive disease entities, it is perhaps interesting to note that 'diffuse Lewy-body disease' has only emerged as a distinct disease in the last few years, despite being possibly the second most common form of neurodegenerative dementia in the elderly (McKeith et al., 1992) and therefore resulting in many thousands of deaths per year.

The technique of immunohistological diagnosis of prion disease has been in use for a number of years and has undergone several improvements since its inception (Hayward et al., 1994). Cases of CJD occurring well before the current BSE epidemic will therefore not have been assessed initially with the same techniques and, in the absence of immunohistochemistry, it is possible that a diagnosis of CJD would not have been reached in some cases. Even though PrP-amyloid deposits can be detected using other histological stains. It will be necessary to carry out a retrospective analysis of archival material from Britain (and elsewhere) in order to set vCJD in the appropriate context.

Having said all this, we recognize that death from unknown neurological conditions in young people is rare and it is likely that all such cases would be viewed extremely seriously. The publication of the clinical description of vCJD does not seem to have resulted in a flurry of retrospectively identified cases and we feel that it is unlikely that, despite the many difficulties involved, as many as five cases of vCJD per year could have been missed between 1990 and 1994.

Does temporal and geographical clustering imply acquired aetiology?

When it was established that CJD and kuru were experimentally transmissible (Gajdusek et al., 1966; Gibbs et al., 1968), it was supposed that these human diseases were acquired by infection. This led to a 'search' for environmental explanations of the occurrence of endemic human prion disease especially where temporal or geographical clustering was perceived. The most persistent of these 'explanations' was the suggestion that the very high incidence of CJD amongst Libyan Jews resident in Israel was due to their supposed excessive consumption of sheeps' brains and eyeballs (Herzberg et al., 1974). It is now established that the excess of cases in this isolated ethnic group is due to the occurrence of a mutation at codon 200 (glutamine \(\rightarrow\) lysine) in the PrP gene leading to large numbers of genetic cases (Gabizon et al., 1992). Similarly, a number of clusters of CJD in Slovakia can now be accounted for by the codon 200 mutation (Goldfarb et al., 1990) rather than, as had been supposed, to a high level of
sheep farming in this area and the implication that sheep scrapie might have been a risk factor.

The populations of most geographical areas show marked socio-economic and micro-geographical stratification that can lead to the startling co-occurrence of cases of prion disease. We and colleagues investigated the lineage of a number of cases of prion disease, associated with a 144 bp insertion in the PrP gene, in four apparently unrelated families resident in southeast England. These families were traced back to three villages within a few miles of each other in West Sussex at the beginning of the 19th century. Analysis of parish records eventually established that these families shared an ancestor (Poulter et al., 1992; Collinge et al., 1992). The occurrence of two or three cases of prion disease within one small area may therefore be attributable to such unrecognized genetic relationships. Three cases of so-called ‘conjugal’ CJD, i.e. CJD in a patient related only by marriage to a relative of a proband, have been reported. In each case the proband was a genetic case which strongly suggests that the conjugal case was a distant, but unknown, relative of the proband and came from a limited number of socially appropriate marriage candidates (Baker et al., 1985b).

In the case of scrapie in sheep it has been held by many that the undoubted familial pattern of occurrence of disease represented vertical (probably maternal) infection (Dickinson et al., 1974). This view was persistently resisted by Parry (1983) who maintained that natural scrapie was a simple recessive disease. Recent re-analysis of the original data on maternal transmission (Ridley & Baker, 1995) casts doubt on the evidence for the deviation from a recessive pattern of occurrence of scrapie, which belief in maternal transmission requires, and molecular analysis of the PrP gene of certain breeds of sheep now indicates that most sheep with natural scrapie are homozygous for certain breed-specific polymorphisms (Hunter et al., 1992). Furthermore, a recent analysis of the occurrence of scrapie in the closed flock of Cheviot sheep maintained since 1960 in Edinburgh has concluded that there was ‘no strong evidence for simple maternal or paternal transmission of disease other than inheritance of PrP genotype’ (Hunter et al., 1996). Although the importance of breeding was well-known in the eighteenth and nineteenth centuries (Parry, 1983), a failure to appreciate the importance of the genotype of the ram leads, even now, to many misinterpretations of ‘outbreaks’ of scrapie. A ram is often used to tup many ewes in one flock and either it, or another ram from the same pedigree stock, may be used in subsequent generations. This can lead to the sudden increase in the number of homozygotes in the flock and the concomitant occurrence of ‘outbreaks’ or ‘clusters’ of cases of scrapie. It is our view that clusters of cases of prion disease should be assessed from a genetic viewpoint before assuming that such an event is necessarily evidence of shared infection.

Other apparent ‘clustering’ of CJD cases may initially suggest an acquired aetiology but may, in fact, arise from ascertainment bias or a failure accurately to calculate the age distribution of the population. The observation that the annual mortality rate from CJD was higher in Paris than in the rest of France led Brown et al. (1983) to argue for ‘random inter-human spread, either direct or indirect, in natural disease transmission’, but may have reflected greater ascertainment of cases in areas with better medical cover or an average older population in cities. In a follow-up analysis of the French data, Raubertas et al. (1989) looked at two types of cluster: space and space-time. There was no evidence of clustering within the city of Paris although there were higher levels of CJD within the city than in the suburbs. No rural clusters were identified and there was little evidence for space-time clustering. In similar studies of CJD in England and Wales between 1970–1984, Cousens et al. (1990) found no evidence of space-time clustering of dates and places at onset although they did find an excess of cases born in London. However, these authors warned that a number of statistically significant relationships in the earlier reports based on data for 1970–1979 were no longer significant when the data were extended to cover 1970–1984.

The recent observation of several cases of CJD in dairy farmers led to speculation that these cases were related to the occurrence of BSE on their farms (Almond et al., 1995). There are various reasons for doubting this conclusion. First, the time between the appearance of a case of BSE in the herd and the farmer’s own illness was, in each case, too short for him to have caught the disease from the animal, though it is logically possible that the farmer could have acquired CJD from the same source as the animal caught BSE. Second, no amount of statistical manipulation can compensate for the fact that the number of these cases is extremely small; a similar apparent excess of cases of CJD in farmers is also seen on the continent in countries which apparently have virtually no BSE (Delasnerie-Laupretre et al., 1995). An apparent excess of cases is also seen in certain professions (e.g. priests and nuns) in which it is difficult to envisage a mode of transmission (Brown et al., 1987). These latter observations probably reflect the difficulty in establishing the appropriate population size and age structure against which to calculate the expected number of sporadic cases. Finally, these farmers presented with an age at onset and clinical picture typical of sporadic CJD. If it is shown that BSE causes vCJD then it is most unlikely that BSE has caused a different presentation in these farmers. If these farmers did contract CJD from an association with BSE, then this casts doubt on the origin of vCJD.

While it is clear that the clustering of vCJD in Britain within the last 2 years is important, claims by the media that clusters have occurred in confined geographical areas within Britain should be regarded with scepticism. The media do not usually differentiate between vCJD and sporadic or genetic CJD which may occur co-incidentally in the same area. Nor do they always report whether cases are biopsy or post mortem confirmed, or are merely clinically suspect (a substantial proportion of which
turn out not to be prion disease), and no account is taken of whether the patients were resident in the same area 5–10 years ago (a reasonable incubation period for orally acquired disease).

How could vCJD be related to BSE?

On August 19th 1996, the coroner recorded a verdict of 'death by misadventure' following the inquest on one of the victims of vCJD, saying that the young man in question had probably contracted the illness as a result of eating some form of BSE-contaminated beef product before 1990, such as beefburgers. But was he right to single out beefburgers?

The only tissues in which infectivity has been detected by bioassay in naturally affected cases of BSE are brain and spinal cord (SEAC, 1995). Introduced in 1984, the 'Meat Products and Spreadable Fish Products Regulations' (whose introduction was unconnected with BSE) prohibited the inclusion of brain, spinal cord and many offals in meat products which were uncooked at the time of sale. This includes most minced meats, sausages and beefburgers purchased from supermarkets and butchers. The major outlets which supply cooked beefburger, though not affected by this legislation, usually provide a product which comprises 100% 'meat' where meat is legally defined as muscle and some offals but not brain and spinal cord. For economic reasons meat used for beefburgers may be imported. To ensure meat preservation, the spinal cord is usually removed from hung carcasses and, in practice, almost all bovine brain and spinal cord has always been rendered for use in animal feed, where it fuelled the BSE epidemic. Some concern has been expressed over 'mechanically recovered meat' (MRM) which, since it comprises residual tissue stripped from bone, could legally be included in uncooked products such as sausages as well as cooked gravies, soups and meat pastes. However, for technical reasons, MRM was not recovered from the head. The SBO Ban of 1989 was intended to prevent inclusion of residual brain and spinal cord in any human food and further modifications have since been made to the legislation to ensure that this requirement is adhered to. At present it seems inappropriate to identify any specific food item as a risk factor for vCJD. The frequent tendency to blame beefburgers results more from the way this food item has come to epitomize all that is thought to be wrong with modern food production than from a serious appraisal of the possible inclusion of infected tissue in this product.

If vCJD is BSE-related, then the most likely source of contamination would be the consumption of the small proportion of bovine brain and spinal cord included in human food prior to 1989, or the inadvertent inclusion of some brain and spinal cord in human food after that time. Or it could suggest that peripheral bovine tissues do contain low levels of infectivity. While it is possible to establish the level of infectivity in bovine tissue by bioassay (transmission to mice or cattle of increasing dilutions of tissue homogenate), extrapolating from this to the risk to humans is not possible. First, even by using primates or transgenic mice it is not possible to model completely the effects of the species barrier that exists between cattle and humans. Second, no experiment designed to show that tissue lacks infectivity, using even a few thousand laboratory animals, could have the same sensitivity as exposure of 50 million people. It is not possible, therefore, to design an experiment which will indicate that no cases will occur in such a large group of people.

Even if it is accepted that vCJD is a new disease, it is possible that it is BSE-related but not via food. For example it could derive from some medicinal or quasi-medicinal product made from bovine brain, although rigorous attempts have been made to prohibit the use of such products. The patients with vCJD are not known to have taken any unusual products. It is also logically possible that vCJD is acquired, but from some unknown source unrelated to BSE.

Can it be proved that there is a link between BSE and vCJD?

Different sources of sheep scrapie injected into mice, and then serially passaged in a panel of mice of various PrP-genotypes, typically produce different patterns of incubation period and distribution of pathology in the brain (lesion profile) (Dickinson, 1976; Dickinson & Fraser, 1979). This has led to the concept of the existence of different 'strains of agent' although the extent to which these data reflect differences confined to the agent rather than encompassing wider aspects of agent-host interaction is open to question (Ridley & Baker, 1996). The same 'strain of agent' has been isolated from several cows with BSE, and from a sheep (of a PrP-genotype not susceptible to scrapie), a goat and a pig all experimentally infected with material from a BSE-affected cow, as well as from three cats, a nyala and a kudu with naturally occurring, but probably BSE-related, spongiform encephalopathy (Bruce et al., 1994). Identification of the 'strain of agent' in vCJD by transmission to the panel of mice should therefore establish whether vCJD is BSE-related, although there may be certain problems. Incubation times can be protracted, so it may take a year or more before results are obtained. It has previously proved difficult to transmit (ordinary) CJD to mice and this may prove so for vCJD. Although 'BSE' has not changed its transmission characteristics on passage through the species mentioned, it does change when passaged through mice of the sincprP  PrP genotype and it has to be supposed that it may have changed on passage from sheep scrapie to cattle (if that is how BSE started) since the 'BSE-strain' has not been isolated from sheep with naturally occurring scrapie. If 'BSE' were to have changed again on passage to humans then these experiments might suggest that vCJD is not BSE-related when in fact it is.

It might be supposed that the transmissibility of 'BSE' to humans can be assessed by transmission of 'BSE' to transgenic mice carrying copies of the human PrP gene. Mice with extra...
copies of the human PrP gene (which express more human PrP than mouse PrP) are susceptible to ‘BSE’ but, rather surprisingly, biochemical analysis indicates that only the mouse PrP enters into the disease process (Collinge et al., 1995). It has not yet been shown that transgenic mice carrying the human PrP gene but lacking the mouse PrP gene are susceptible to ‘BSE’. We should remember that, at present, a causal relationship between BSE and vCJD is not established.

Conclusion

At present, a small number of cases of a new variant of CJD has occurred in Britain and one in France. The number has not been sufficient to produce a significant increase in the level of CJD in Britain. If BSE had caused (ordinary) CJD, this additional number of cases would not have been noticed. The evidence that these cases are related to BSE is circumstantial and it may be a year or so before experimental data can establish such a link. Meanwhile, the beef market in Britain and the rest of Europe has collapsed and public confidence in scientists and politicians has been severely dented. The epidemic in cattle has been extremely difficult to handle because an incubation period of several years meant that it was well-established before the first cases of BSE were identified. Decisions had to be made on the basis of sparse evidence because further research would often have taken several years to complete. Extrapolating from data in laboratory animals to cattle or humans is fraught with difficulties because prion disease behaves differently under different conditions. Further problems have arisen from a cultural misunderstanding between scientists and policymakers. Scientifically, the risk of transmission of ‘BSE’ to humans was calculated to be ‘extremely small’ especially after the brain and spinal cord had been banned, although this risk was not quantified. But when an extremely small risk is multiplied by a very large number of people exposed (the population of Britain is ~ 50 million people) a few cases might occur. From a public health point of view the possible occurrence of a few cases has proved to be politically unacceptable. The BSE epidemic in cattle is now all but over and the greatest risk to humans occurred some years ago. But faced with political pressure from our European partners, the Government may have had little option but to instigate a slaughter policy for cattle even though this may have little impact on human health.

There can be little doubt that vCJD is a newly recognized prion disease. That, so far, it is confined largely to Britain (with one case in France) and that all the cases have occurred in the last 2 years, provides the basis of SEAC’s conclusion that these cases are likely to be linked to BSE. There are, however, a few sources of doubt. Attempts to transmit BSE to transgenic mice carrying only the human PrP gene have not yet been successful. Transmission experiments have not established that BSE is more infectious to primates than is scrapie (Baker et al., 1993). If infection from cows is a risk but from sheep is not a risk, this difference could lie in the way in which we make products from these animals (e.g. age of animal used) rather than the ‘virulence’ of the agent. No cases of (ordinary) CJD have been attributed to infection from any animal source. Although vCJD was recognized because of its occurrence in young people, there is no reason to suppose that only young people will be at risk of BSE-related disease so it is possible that vCJD is merely an extremely rare, but long-standing, variant in young people. Finding a case of vCJD which predates the BSE epidemic or which comes from a distant part of the world would support this. If the number of cases of vCJD begins to rise rapidly, this will confirm the acquired nature of the illness but, so far, no large increase has been reported. Strain-typing experiments may be able to provide a definitive answer, but they may be inconclusive. At the present time, neither we nor anyone else can be certain that vCJD is BSE-related and, indeed, one of us believes that these cases are probably BSE-related while the other is uncharacteristicallyagnostic. Many years of working in this field has taught us that the world of prion disease is full of surprises.

Note added in proof. Since this review went to press, Collinge et al. (Nature 383, 685–690, 1996) have reported that the abnormal protein, PrPSc, varies in its molecular structure depending on the genotype of the host and whether or not the disease is sporadic or acquired. The prion protein from the vCJD is indistinguishable from that found in cattle with BSE and other species to which BSE has been transmitted and differs from the prion protein found in sporadic and iatrogenic (acquired) CJD. We believe that these data provide strong evidence that vCJD is BSE-related.

References


