BSE and human prion disease

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Prion disease is now the preferred collective term for the spongiform encephalopathies, because it emphasises their common molecular pathogenesis (Prusiner, 1993). Prion disease includes Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cows. In their editorial, Fleminger & Curtis (1997) describe a new variant of CJD and the suspicion that it might be related to BSE. Here, the evidence for and against BSE transmission to humans is summarised in the light of increasing evidence that the new variant CJD cases are indeed the result of BSE. The recently reported *in vivo* diagnostic tests for prion disease are also discussed.

THE BSE EPIDEMIC

It is thought that BSE arose because of changes in the way animal remains were rendered to make high-protein animal feed in the UK (Wilesmith *et al*, 1991). The result was that from about 1980 until 1988, when ruminant feed was banned, its contamination by scrapie-infected sheep carcases and perhaps by pre-existing cases of BSE was no longer being neutralised. In other words, ovine and bovine abnormal prion protein (PrP^{Sc}) was not destroyed in the feed that was being given to cattle. Dairy herds rather than beef herds were particularly affected because of the extent and duration of feed supplements they received. A vicious cycle was set up once an increasing number of BSE-infected cows were included in the rendering process. BSE was therefore an inadvertent demonstration of the dietary acquisition of prion disease between and within species, a mode of transmission already known from kuru (human prion disease spread by cannibalism) and intentional experiments (Gibbs *et al*, 1980).

By mid-1996 there had been 160 000 confirmed cases of BSE in the UK, although, because of the long incubation period and under-reporting, the actual number of infected cows is estimated to be close to a million (Anderson *et al*, 1996). About half of these animals will have entered the food chain before the 1989 Specified Offals Ban removed the infectious parts of the cow, which are mainly the brain, spinal cord and ileum.

In this context, the scientific and public concern about BSE is understandable (Table 1). During the 1980s, eaters of British beef products were the unwitting participants in a second unplanned experiment: whether or not humans can catch BSE through diet (Harrison & Roberts, 1992). We are living through the incubation period of the experiment, which kuru and iatrogenic CDJ data suggest is 5–15 years (with wide confidence limits). To set against the reasons for concern, there is a wealth of reassuring epidemiological data about scrapie, to which we have been exposed for generations and which shows no evidence of an association with human prion disease; indeed, the only established non-genetic risk factor apart from iatrogenesis is a history of psychosis – a finding of unknown significance (Wientjens *et al*, 1996). Further reassurance comes from the ineffectiveness of oral transmission between species. It requires huge doses and is many-fold less efficient than the intracerebral route, presumably reflecting the difficulty with which PrP^{Sc} gets from the gut lumen to the central nervous system. Thus, despite the theoretical arguments for transmissibility, in practice no one may have been exposed to enough bovine PrP^{Sc} to develop disease. Calculations regarding infective doses are generally reassuring, albeit subject to doubt

Table 1 Reasons for and against proposing BSE transmission to humans

For	Against
Infectivity survives cooking	Extensive epidemiological studies have never shown a link between exposure to scrapie and CJD
The unequivocal oral transmissibility of prion diseases. Specifically, BSE arose from cows eating contaminated animal remains, and has in turn been transmitted by diet to mice and goats	Dietary spread of prion disease *between* species is very inefficient and would require unfeasibly large doses since most of the cow, and dairy products, are not infectious
Until 1989 infectious offals were included in many meat products, such as sausages, burgers and pies	Since 1989, all potentially infectious material has been banned from the food chain
Prion-diseased animals are infectious before symptoms emerge. Many asymptomatic but BSE-affected cows will have entered the food chain	Prion disease has not been seen in primates fed for life on ruminant-derived protein
A single meal of 1 g BSE-infected brain can give a cow BSE	There has not been a large outbreak of prion disease in pets, although pet food contained much offal and the shorter incubation period in these animals means that any epidemic would already be apparent
Spot checks showed one-third of abattoirs not adhering to the offal ban regulations	The number of CJD cases in the UK has not increased in recent years. The incidence remains similar to that in the rest of Europe and the USA, where there has been no BSE epidemic
A possible cluster of CJD cases in dairy farmers	

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over orders of magnitude (Almond *et al*, 1995; Dealler, 1996).

EVIDENCE THAT BSE HAS CAUSED HUMAN PRION DISEASE

The presence of evidence both for and against the possibility of BSE transmission has meant that neither unequivocal reassurance nor meaningful attempts at human disease risk quantitation have been possible. Instead, the adjectives chosen by scientists to describe their opinion on the matter, terms like 'remote' and 'negligible' and the nuances between them, have taken on uncomfortable significance. However, this phase of the BSE story is coming to an end. It appears likely that BSE has led to human prion disease; the adjectives will now be used to describe the probability that the current handful of cases will turn into an epidemic, and how large it might be.

The crucial shift in this balance of probabilities came from the report of the new variant CJD cases. On the basis of the subjects' age and unusual clinical and pathological features (Table 2), Will *et al* (1996*a*) concluded that "exposure to the BSE agent is perhaps the most plausible interpretation" of the new variant of CJD, even though there had been nothing remarkable in the dietary and occupational histories of the individuals affected. Commenting on the data, an American expert, who had been dismissive of the risk from BSE (Almond *et al*, 1995) admitted that "it now appears I was wrong" (Brown, 1996). Nevertheless, the evidence was still circumstantial. Subsequently, however, the evidence linking new variant CJD to BSE has become stronger. First, the florid plaque-type neuropathology, already noted to be more like that of kuru than other human prion disease, was shown to occur in BSE-infected monkeys (Lasmézas *et al*, 1996). Then, more direct evidence supporting a BSE aetiology for new variant CJD was provided (Collinge *et al*, 1996). Brain tissue affected by different types of prion disease was homogenised and underwent gel electrophoresis and Western blotting. These techniques allow proteins to be separated by molecular size, and individual proteins to be identified using antibodies. Application of anti-PrP antibodies led to three protein bands being detected, reflecting PrP molecules in different glycosylation states (i.e. different numbers of sugar moeities attached; Parchi *et al*, 1996). Collinge and co-workers observed four patterns in terms of the relative intensity of the bands. Types 1 and 2 were seen in typical (sporadic) CJD and in iatrogenic cases caused by a dura mater graft. Type 3 occurred in CJD resulting from peripheral (intramuscular) iatrogenesis. Crucially, all the new variant CJD cases had a distinct pattern – Type 4. Moreover, the Type 4 pattern was shared by brain tissue from BSE-affected cows and from other species infected with BSE. In other words, the molecular signature of prion disease in BSE and new variant CJD was the same, but was different from all other forms of human prion disease. The authors concluded that, although additional experiments were needed for a definitive answer, the result was "consistent with the hypothesis that new variant CJD results from BSE transmission to humans" (Collinge *et al*, 1996).

Table 2 Comparison of typical CJD and new variant CJD

Variable	Typical CJD	New variant CJD
Age at onset	50–70 years	19–39 years
Presentation	'Neurological' – dementia	'Psychiatric' – depression, personality change, dysaesthesia
Clinical course	Rapidly progressive, death in 6–12 months	Insidious onset, slower course (7.5–24 months)
PrP codon 129[1]	Usually MM or VV	All are MM
Pathology	Scanty, punctate PrP deposits	Florid PrP amyloid plaques
PrP bands[2]	Types 1 and 2	Type 4

1. See Table 3.
2. See text ('Evidence that BSE has caused human prion disease').

PREDICTIONS

Fifteen new variant CJD cases have now been identified. Mathematical modelling suggests that there will eventually be between 80 and 80 000 cases, the uncertainty reflecting the unknown length and variability of the incubation period (Cousens *et al*, 1997). In the meantime, predictions may be made as to which individuals are at particular risk (Table 3; Collee, 1996).

There has been much progress towards an *in vivo* diagnostic test to replace brain biopsy (Collinge, 1996). A protein called 14-3-3 (unrelated to PrP) can be measured in cerebrospinal fluid and differentiates prion disease from other dementias with a high sensitivity (98–99%) and specificity (96–99%; Hsich *et al*, 1996; Lee & Harrington, 1996; Will *et al*, 1996*b*). An ideal diagnostic test would also be able to detect presymptomatic cases, work on accessible peripheral tissues, and distinguish new variant CJD from other forms of human

Table 3 Factors which may affect individual susceptibility to BSE-acquired prion disease

Exposure to BSE PrP^{Sc}	Genetic factors	Other factors
Through diet – amount and type of beef products eaten in the 1980s	PrP gene polymorphism. At codon 129, amino acid can be methionine (M) or valine (V). MM homozygotes (~38%) are most susceptible, VM heterozygotes (~50%) are least susceptible	Younger people may be more at risk
Through occupation – abattoir workers, farmers, vets, etc. Abattoir workers are at additional risk via conjunctival entry		Gastrointestinal permeability and absorption of intact PrP^{Sc}
		Efficiency of PrP^{Sc} transport and of PrP^{c} conversion once in the body

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prion disease. The 14-3-3 test is unlikely to satisfy the latter two criteria, but another diagnostic approach may meet all three objectives. It is based on the finding that PrP^{Sc} can be detected in tonsils, both in animals prior to symptoms (Schreuder *et al*, 1995) and in a new variant CJD patient (Hill *et al*, 1997); importantly, her tonsillar PrP^{Sc} had the characteristic Type 4 banding pattern. Thus, tonsillar biopsy may allow diagnosis of overt and pre-clinical new variant CJD. (In principle, the same techniques could be used after routine tonsillectomy or at autopsy to determine the population frequency of new variant CJD-type tonsillar PrP^{Sc}, as a crude estimate of the number of people who have eaten and retained BSE-infected material. However, the data could not be used to predict how many new variant CJD cases will occur, since the chances of a 'tonsil-positive' person ever developing the disease are unknown.)

There are no imminent prospects for therapy. Proposals that the PrP gene could be inactivated to prevent *de novo* synthesis of PrP^{Sc} are less attractive given that transgenic mice lacking PrP are not entirely normal, as had originally been thought (Sakaguchi *et al*, 1996; Tobler *et al*, 1996). Alternatively, one could try to prevent environmentally acquired PrP^{Sc} ever getting to the brain or, if this has already occurred, interfere with the corruption of endogenous PrP^{Sc} molecules into more PrP^{Sc} (Fleminger & Curtis, 1997, Fig. 1). There is an urgent need for information about the molecular basis of these processes if prion disease is to become treatable.

By the end of the decade, it should be clear whether BSE has or has not resulted in a significant outbreak of human prion disease, and whether recriminations or relief are in order (Gore, 1996). Regardless, there are many lessons to be learnt from reconstruction of the BSE saga, including the consequences of deregulation of the food industry and the government's role in the crisis (Anonymous, 1996*a*,*b*; Maddocks & Dealler, 1996; McKee *et al*, 1996), what constitutes evidence of causality, and how relative risk is portrayed to and by the media (Anonymous, 1996*c*; Baker & Ridley, 1996).

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It is also worth noting that much of the understanding of prion diseases has come from research funding at a time when the diseases were scientifically intriguing but had little public health significance. The costs of that research have proved to be a drop in the ocean compared with the costs of BSE, which are running into billions of pounds.

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