BSE did not cause variant CJD:
an alternative cause related to
post-industrial environmental contamination

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Summary The new prion diseases that have emerged in the last 15 years are bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (variant CJD). Although initially confined to the UK, these diseases have recently emerged in other European countries. The accepted cause of the human disease is that BSE spread from cattle to humans by the consumption of infected beef. However, the evidence that supports this is very thin. This article describes this evidence and lists a series of hypotheses concerning the cause of both BSE and variant CJD. The final hypothesis is based on recent evidence linking prion diseases to environmental factors including manganese. High environmental availability of manganese is associated with the prevalence of those prion diseases not linked to BSE. Therefore it is quite possible that BSE and variant CJD have emerged as a result of manganese-rich industrial pollution that has only occurred in the last century. © 2001 Harcourt Publishers Ltd

HYPOTHESIS 1: THE BEEF HYPOTHESIS

The nature of prion diseases (transmissible spongiform encephalopathies) has long been disputed. It is now almost certain that these diseases result from conversion of a normal cellular protein, the prion protein (PrP\textsuperscript{c}) to an abnormal aggregating, amyloidogenic protein (PrP\textsuperscript{sc}) (1). This abnormal protein has been suggested to be a ‘prion’ or infectious protein (2). That infectivity in these diseases is only associated with this protein is not proven. Indeed, for the majority of these diseases infection is not an issue. The majority of prion diseases such as scrapie in sheep, chronic wasting disease (CWD) of deer and elk and the human disease Creutzfeldt–Jakob (CJD) disease all occur with no association to either direct contact with infectious material or eating of infectious material (1). In humans the other forms of prion disease are mostly inherited diseases such as Gerstmann–Straussler–Scheinker syndrome or fatal familial insomnia (1,3,4). In terms of human prion disease the only ‘certain’ cases of infection are the disease Kuru, caused by eating of infected human brain, and the iatrogenic CJD cases associated with infected material from humans being injected or implanted in humans (5).

Until the suggestion that the disease bovine spongiform encephalopathy (BSE) passes to humans in the form of variant CJD there was no evidence that animal disease can cause human prion disease. Indeed, the existence of a species barrier (6) that prevents transmission of clinically manifested disease between individuals of different species, in experiments, suggests that transmission of disease from cows to humans is highly unlikely or impossible. Indeed, transmission of scrapie to humans is accepted as not occurring.
The origin of BSE

Although the BSE crisis in the UK is apparently approaching an end, there is now renewed concern of similar crises in other countries, and France, Portugal and Switzerland have substantial numbers of cattle developing this disease. The first and most immediate concern about these new epidemics is related to the cause of BSE. BSE is claimed to have occurred as a result of a subtype of scrapie infecting cattle through ingestion of contaminated offal (7). There is little doubt that the recycling of the remains of cattle back into their own food supply escalated the BSE epidemic to the point where there were tens of thousands of cases in a single year. This is testified to by the effectiveness of the food ban that came into place once it was realized that the offal could have caused the disease. However, despite continued investigation the origin of BSE is not certain.

BSE does not resemble any strain of scrapie. BSE resembles variant CJD more than it does any other form of sporadic prion disease. Indeed BSE has just appeared spontaneously. Furthermore, in other European countries BSE continues to appear spontaneously. However, as has been demonstrated, prion disease can occur in a subclinical form that does not lead to apparent disease. It is not possible to know how far back into the past subclinical BSE has existed or to what extent. In terms of the virtually Europe-wide threat of BSE, which can no longer be proven to be related to imports of UK cattle, it is almost impossible to prove that BSE is related to ‘infection’.

The widespread nature of BSE now suggests that it should be considered a sporadic disease, like scrapie. This view should be adopted as long as there is no evidence that the majority of European cases of BSE can be linked to imported UK cattle or feed.

The weak link between BSE and variant CJD

At the time of writing the article the number of French cases of variant CJD stands at four. This is considerably less than the number of cases of vCJD in the UK, which is now approximately 100 cases. However, as a ratio to the number of cases of BSE that France has had, it is relatively high. Whereas the ratio of variant CJD cases to BSE cases for the UK is approximately 1:2000, that for France is 1:50 or less. It is currently assumed that those cases of variant CJD in France arose because of the eating of UK beef. However, this remains a matter of pure speculation and if the number of variant CJD cases in Europe increases in a similar manner for the next 5–10 years, it will become almost indisputable that those cases were caused by something inherent to France. It is not possible that the French eat more UK beef or cattle products per head of population than the British do.

At the centre of the whole controversy over BSE and variant CJD is the hypothesis that variant CJD is caused by children eating contaminated beef from BSE-infected cattle. This hypothesis is seen almost as ‘gospel truth’, because of the way it is repeated by scientists and lay people alike without the slightest knowledge of the evidence for or against it.

Scientific proof that BSE causes variant CJD rests upon the fact that BSE was first diagnosed 10 years before variant CJD was diagnosed. However, there is no proof of how long subclinical BSE and variant CJD existed in the UK before this time. The report of an individual diagnosed with variant CJD at 74 years-of-age makes it quite possible that individuals could carry the disease for 50 or more years. This is known to be the case for the human prion disease Kuru. Incubation time for prion disease, i.e. the length of time they can be subclinical following ‘infection’, is related to the lifespan of the species. The lifespan of humans is much longer than that of cattle. Therefore it is quite possible that the 10-year gap between detection of the first human cases and the first cases in cattle is simply due to differences in incubation time. Therefore, the temporal relationship between BSE and variant CJD only coincidently supports the notion that BSE caused variant CJD, and as such is not strong evidence.

The evidence other than this comes from research using mouse models and analysis of subtypes of abnormal prion protein. This supporting evidence is related to four papers published in high-ranking journals (8–11). The sheep disease scrapie has various ‘strains’. These strains differ in that they have reproducible and characteristic patterns of manifestation when transmitted to specific strains of mice. These characteristics include incubation time, localization of PrPSc, localization and extent of neuronal loss, vacuolation and gliosis. A specifically modified mouse expressing the human prion protein can be infected with human prion disease such as sporadic CJD. When this occurs no particular strain emerges. Individual cases of CJD produce different profiles. However, variant CJD cases produce a single profile in mice expressing the human prion protein. When the same mice are infected with BSE, the same profile as variant CJD emerges.

PrPSc has three forms depending on glycosilation. PrPSc is identified as such by digestion of brain extracts with proteinase K. The electrophoretic mobility of these proteinase K-digested glycoforms produces a pattern which is characteristic for either the strain of scrapie or the subtype of sporadic CJD. Similarly, variant CJD has a specific pattern of glycoforms that is unlike those of sporadic CJD. However, the glycoform pattern produced by variant CJD and BSE injected into mice is similar.

No matter how convincing this might sound, these results do not demonstrate that BSE caused variant CJD. If the results as described are accepted they simply show...
that BSE and variant CJD are the same ‘strain’ of prion disease. However, it is not really clear what a strain is or how it is caused. Furthermore, the whole idea of ‘strains’ is based on prion disease in mice. Mice have no known naturally occurring prion disease. Therefore, the disease the mice get is not BSE and it is not variant CJD. Just as many mouse models of human disease fail to recapitulate the details of other human disease, these mouse models fail to recapitulate the details of human prion disease.

The incubation time for these diseases in mice are measured in months, while in humans they are measured in years or even decades. The devil is always in the details and although it is ‘comfortable’ to assume that similarities in the disease profiles of mice infected with either BSE or variant CJD imply that these diseases are ‘similar’, the fact is, a mouse is not a cow and it is not a human. Condensing a disease that takes 20 years in a human or 6 years in a cow to a few months in a mouse will certainly abolish any subtle differences that would otherwise distinguish them. One such difference is the occurrence of plaques. Plaques are spherical aggregates of PrPSc that can be detected in some forms of prion disease. Variant CJD is characterized by plaques, while BSE is not.

HYPOTHESES 2 AND 3: VARIATIONS ON THE LINK TO BSE

The prevailing hypothesis that BSE was transmitted to humans by eating beef is fixed as the only way that the disease could have spread. However, if one discusses this with an expert in the field then the answer is that it can only really occur if the meat is contaminated with large amounts of nerve tissue. Currently, assays for the infectious agent of BSE. There is no evidence that a piece of meet cut from a BSE-infected cow contains PrPSc. When examining the udders of BSE-infected cows to see if the disease-specific protein is present. Despite the millions of pounds sterling spent on research in the UK each year under the assumption that variant CJD is caused by BSE, nobody has done or has been able to do the critical experiments to verify what everyone seems to take for granted.

If one agreed with the idea of transmission of BSE to humans then one should consider the evidence which suggests that the infectious agent can be transmitted from subclinically infected animals to other animals causing clinical disease in the recipient (13). Possibly humans have been eating BSE-infected cattle since the end of the last World War. If cows exist with subclinical BSE then clearly humans probably also exist without clinical symptoms of variant CJD, but carrying the disease. The possibility of transmission from mothers to children has not been explored thoroughly or taken seriously. Transmission of BSE from cows to calves could explain why most cattle with the disease were young and not old cows. Furthermore, in utero transmission for humans also remains a possibility.

These variations on the standard story of the origin of BSE seem plausible, but they too are not considered by the experts supporting the standard BSE-infected meat cause for variant CJD. This single-minded assumption that only beef can be the cause is in itself dangerous. The assumption that variant CJD has to be caused by BSE is far worse.

THE FAILURE OF THE BEEF HYPOTHESIS

Currently we are left with no proof that variant CJD was caused by BSE. Current scientific endeavour has only produced the scant body of evidence described above. Added to this must be the blatant lie spread, particularly by the media, that beef from BSE-infected cattle carries the infectious agent of BSE. There is no evidence that a piece of meet cut from a BSE-infected cow contains PrPSc. When such a critical piece of evidence is missing from the picture one is left to wonder why no other explanation other than BSE as the cause of variant CJD is considered. Even the recent inquiry into BSE (The Phillips Inquiry), although taking statements from people who disagreed with this theory, did not conclude that other possibilities should be investigated.

Perhaps variant CJD was caused by BSE, but if there is another cause and all endeavours to investigate any are discredited as being illogical or ridiculous, then no advance to prevent or treat the variant CJD will be achieved beyond the serendipitous result of trial and error. Scientific method, as opposed to political bluster, suggests that theory should be tested by refutation. A credible theory can only be supported when the evidence opposing the theory is compared to that supporting it and the supporting evidence found to be greater and more credible that the refuting evidence. Therefore, the logical
way to test the validity of the theory that BSE caused CJD is to investigate if there is evidence for an alternative explanation.

**AN ALTERNATIVE HYPOTHESIS IS NEEDED**

In searching for an alternative theory of the cause of BSE and variant CJD one needs to examine the origins of the disease and parallels between them, or with other diseases. Currently, there are very few cases of variant CJD. Thus an analysis of variant CJD is difficult. However, there is some evidence of clustering of cases. Although the two known clusters (Quineborough and Doncaster) contain only a handful of cases, even this small evidence suggests there might be a link to some environmental factor. However, because of the small numbers it could simply be that the co-localization of these cases is a misleading coincidence.

Therefore, analysis of BSE might provide a clearer insight into an alternative explanation for the cause. The problem here is that it remains to be determined whether BSE is a new true sporadic disease or not. However, as cattle born after the Feed Ban of the late 1980s still contract BSE then it is becoming more likely that BSE is a sporadic disease, especially as Europe now has increasing incidence of BSE that cannot be linked to UK livestock. The alternatives are that BSE can be transmitted between mother and calf either in utero or via milk. However, there is little evidence that such transmission is successful in other forms of prion disease. A further alternative is that the Feed Ban has been ineffective and that contaminated material from BSE-infected animals is still entering the food-chain. This again is almost impossible to determine but it seems unlikely because the amount of infected material that is entering the food-chain of cattle must be truly minuscule when compared to the amount of infected offal that was deliberately fed to cattle before the height of the BSE epidemic. The incidence of BSE has not decreased to the same degree as the reduction in the use of animal offal as a feed for cattle.

Gauging the original distribution of BSE is difficult, as BSE was clearly magnified in incidence by the feeding of offal from BSE-infected cattle. However, if we assume that BSE is a sporadic disease then we can study other sporadic prion diseases to determine if there is some environmental link to the incidence of these diseases. However, most sporadic prion diseases appear to have existed for a long time. CJD was first described in the 1920s but there is evidence for it in the 1800s and similarly scrapie was first described in early records as well. In our scientific age it is very difficult for such diseases to go unnoticed, therefore BSE is a novel disease which has emerged at some time in the 20th Century.

It has long been known that scrapie does not arise at random, but certain farms or certain regions have higher incidences than others do. In Iceland scrapie is very high in particular valleys while other regions show low incidence. This is also true of the UK and elsewhere in the world. Surprisingly, this phenomenon cannot be associated with particular strains of sheep and when the sheep on a particular scrapie-prone farm are slaughtered and the vegetation on that farm replaced, new stock develop scrapie with the same incidence. Additionally, CWD the disease of deer in the USA, also shows a regional distribution. Sporadic CJD has also been noted to have higher incidences in certain localities around the world, such as regions of Slovakia and Crete. These findings suggest that there is a possible influence on the occurrence of prion disease.

**HYPOTHESIS 4: A POST-INDUSTRIAL ENVIRONMENTAL CATASTROPHE**

Mark Purdey has speculated that prion disease could be linked to either organophosphate pollution (14) or imbalances in the metal-ion content of foodstuffs, or a combination of both (15). Certainly organophosphates can increase the expression of PrPc as demonstrated by cell culture experiments (16), and increased PrPc expression shortens the incubation time for prion disease (17). However, it is difficult to determine at present if organophosphates play a part in the aetiology of any prion disease. A link between metals and prion disease is more supportable. Recent evidence suggests that PrPc can bind either copper or manganese (18,19). However, on binding manganese the protein is more likely to change its secondary structure from one resembling PrPSc (19).

Mammals are the only animals known to be susceptible to prion diseases. How does mammalian PrPc differ from other animals such as birds and reptiles? Currently, there is not a lot of evidence for strong differences in structure. However, mammalian PrPc contains an octameric repeat region with four or five repeats of a sequence containing histidine. Other animals have a hexameric repeat region with each repeat also containing histidine (20,21). These repeat regions are the site at which copper binds to form the holo-form of the protein. As the nature of the metal occupancy of this region can influence the secondary or higher structure of the protein (22,23), then perhaps the change from hexameric to octameric repeats in evolution has disadvantages in that it makes the protein more susceptible to misfolding as a result of aberrant metal incorporation.

Stabilization of copper incorporation might prove to be therapeutic or preventative. Protecting cattle or sheep against prion disease by agents that facilitate copper incorporation might emerge as a way to abolish BSE.
However, while the relevance of metals to this disease is still disputed and blatantly ignored by government funding agencies because of the arrogant views of so-called experts, then no advance in the understanding of prion disease is presently likely.

So what evidence is there that there is a link between metal imbalance and prior disease? Mark Purdey has analysed the metal content of soil and plant samples from regions where sporadic prion diseases have high incidences and compared these to neighbouring regions of low incidences (15). His findings consistently highlight a higher manganese content and a decrease in metals such as copper and selenium (15). Therefore, there is already a suggestion that high manganese might be a causal factor. However, this on its own is as circumstantial as the evidence of the link between BSE and variant CJD. This evidence does not prove that manganese incorporation into PrPc causes conversion of PrPc into PrPSc (19). Cells cultured in media with high levels of manganese in place of copper can cause conversion of PrPc into PrPSc (19). Cells dissolved in the medium show increased expression of proteinase K resistant PrP (19). Whether this protein is ‘infectious’ is uncertain. However, there is no evidence that sporadic prion disease results from infection. In vitro conversion assays suggest that this abnormal PrPc can convert recombinant PrP to an abnormal form (D. R. Brown, unpublished data), which is proteinase K-resistant. Such an effect has been demonstrated for PrPSc, implying that the abnormal PrP from the manganese-treated cells is equivalent to PrPSc in many ways.

Studies of people who live in regions of Slovakia high in industrial contamination of manganese have shown that the population have much higher levels of manganese in their bodies. These same regions of Slovakia also have a higher than usual incidence of sporadic CJD. Thus in this one region of the world there is already evidence of a link between manganese and CJD. This evidence does not prove that manganese incorporation into PrPc causes prion disease, however, high environmental manganese might increase the risk of CJD.

Evidence is now emerging concerning the metal content of the brains of animals and humans with prion disease. The most alarming finding is preliminary evidence from two independent groups that CJD patients have a 10-fold increase in the levels of manganese in their brains (24, 25). This evidence implies that high brain manganese might be a specific hallmark of these diseases. Rapid detection of high brain manganese is possible due to its paramagnetic qualities. Thus MRI could be used to make a preliminary diagnosis of prion disease of patients, possibly early after the onset of symptoms.

What remains to be determined is what comes first: high manganese or prion disease? It is always possible that the accumulation of abnormal PrP might trap manganese in the brain. It is also possible that the high manganese might be due to an increase in the antioxidant mitochondrial manganese superoxide dismutase. Nevertheless, these findings clearly indicate that disbalance in metal ions in the brains is linked to prion disease.

It is very difficult to investigate the link between the incidence of a disease and an element found ubiquitously in the environment. Manganese is everywhere and it is ridiculous to suggest that manganese on its own is a danger to human health. What needs to be determined is in what form or in association with what other factors manganese might exacerbate or trigger prion disease.

One possibility is that mining and use of manganese in various industrial processes that have only begun in the last century might deposit in the environment a form of manganese which is readily absorbed and can initiate pathological changes such as prion disease.

I propose that the new prion diseases are post-industrial phenomena which will spread through the world in relation to the time and extent of industrialization in the countries of the world. Europe is therefore a logical starting point for the emergence of such diseases, as is Japan. Countries in which industrialization is low in comparison to the size of the country, e.g. Australia or Canada, may never see these new prion diseases. Indeed, in support of this idea, Australia has very low scrapie, which although it is not a new prion disease is a sporadic disease that might be linked to environmental factors. Farming areas in Australia are widely removed from areas of industrialization. CJD exists at the same incidence as elsewhere in the world but most people live in industrialized areas of Australia whereas sheep do not. Additionally, those countries that lie in the path of airborne pollutants from other nations (such as Iceland and the UK) might also show increased incidents of new prion disease or sporadic prion disease because of this pollution. It is likely that Japan will be the first country outside Europe to see BSE or variant CJD emerge within its borders.

**CONCLUSION**

The truth behind the cause of BSE and variant CJD remains unresolved. However, current data fails to conclusively prove the accepted hypothesis that BSE was derived from scrapie and that variant CJD arose from BSE. Fortunately, variant CJD is still low in incidence but as a result there is little that can be clearly concluded about its cause. BSE is similarly surrounded in mystery as regards its origins. However, the pan-European occurrence of BSE suggests that alternative explanations must be considered. Although environmental damage due to industrialization sounds a rather vague cause for BSE it is a sufficiently broad base on which to launch well planned investigations into the cause of these diseases.
New insights into sporadic disease points towards manganese as a possible factor to consider. However, if the current unfounded smokescreen of certainty over the origin of variant CJD and BSE is maintained, we may allow a worldwide catastrophe to develop and, like the UK BSE epidemic, any solution may come far too late.

REFERENCES