

The origin of bovine spongiform encephalopathy: the human prion disease hypothesis



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The cause of the original case or cases of bovine spongiform encephalopathy (BSE) remains an enigma. Sheep scrapie or a previously undetected sporadic bovine transmissible spongiform encephalopathy (TSE) have long been considered as candidates, but no convincing evidence to support these proposals has come to light. We present a new theory, with three related hypotheses: (1) that BSE was acquired from a human TSE (prion disease); (2) that the route of infection was oral, through animal feed containing imported mammalian raw materials contaminated with human remains; and (3) that the origin was the Indian subcontinent, from which large amounts of mammalian material were imported during the relevant time period. Human remains are known to be incorporated into meal made locally, and may still be entering exported material. Further investigations are needed into the sources of animal by-products used in animal feed manufacture, and into the transmissibility of human TSEs to cattle.

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Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that affect human beings and several other mammalian species. TSE pathogenesis involves the modification of a normal cellular protein, known as prion protein (PrP^c), into a pathogenic form designated PrP^{Sc} or PrP^{res}. TSEs can be genetically determined, acquired via TSE-infected material, or sporadic. No cattle TSE had ever been recognised until 1986 when the first case of BSE was reported in Britain. The incidence of the disease rapidly reached epidemic proportions,¹ peaking in late 1992. More than 180 000 cases have been recorded in the UK. BSE has also subsequently been detected on a much smaller scale in 19 other European countries, Israel, Japan, and recently Canada and the USA. Exposure of cattle to the BSE agent had almost certainly begun by 1981,¹ and unrecorded cases probably occurred before this year,^{2,4} in the 1970s or earlier.⁵

The emergence in 1995 of a new type of human TSE, now designated variant Creutzfeldt-Jakob disease (vCJD), led to the hypothesis that vCJD was probably a direct result of BSE transmission to man. More than 150 cases of vCJD have been diagnosed in the UK. There have also been four patients in Ireland, Canada, the USA, and Japan, who were potentially exposed in the UK, and a further nine patients in France and one in Italy for whom no infection in the UK could have occurred.

Most authorities agree that the main route of propagation of the BSE epidemic was via the recycling of contaminated remains of BSE-infected cattle in the manufacture of cattle feed.^{1,2,4,5} However, the source of the original bovine case or cases remains controversial. The cause could have been intrinsic (arising spontaneously in individual cattle) or extrinsic (acquired by transmission from another species; table).

An intrinsic event, the mechanism favoured by some experts,⁵ could have been a somatic or germ-line mutation of the prion protein gene *PRNP*, or a post-translational conversion of PrP^c to PrP^{res} (table). However, no genetic TSEs in cattle have ever been identified. A retrospective histopathological survey of

cattle brains archived before 1985⁷ found no evidence of earlier TSE cases to support the existence of a sporadic strain in cattle. In countries where BSE cases have been detected over several years, the incidence over time has generally shown a progressive rise or rise and fall,⁴ suggesting an acquired cause. Large-scale screening of cattle in different countries has brought to light the existence of bovine TSE strain variations⁸ that could be sporadic. However, it seems more likely that these cases were acquired.⁹ The atypical strains were found in about a quarter of the cases that were investigated, and it is unlikely that sporadic strains occurring with this frequency could have escaped detection before.

To investigate a possible extrinsic cause of BSE, two categories of evidence need to be considered (panel): potential exposure to infection, and strain similarities between the putative source TSE and BSE. The most widely favoured theory is that BSE originated from transmission of sheep scrapie to cattle.^{1,2} It is well known that sheep products were incorporated into cattle feed. However, there is no satisfactory explanation for why BSE did not appear earlier, since: scrapie has been endemic in Britain for at least 200 years; meat and bone meal containing sheep material had been fed to cattle for as long as 70 years;⁸ and scrapie infectivity must have entered cattle feed in substantial quantities. One proposal was that feed only became infectious after the phasing out in the 1960s to 1980s of a solvent extraction step during rendering. However, experimental studies have shown that solvent extraction makes little or no contribution to the modest reduction of scrapie (or BSE) infectivity produced by rendering.¹⁰

Support for the scrapie theory could come from identification of a scrapie strain similar to BSE (webappendix). Different prion strains were originally defined on the basis of incubation periods and distribution of neuropathology (lesion profile) following transmission to selectively bred mice (conventional strain typing). Prion strains can also be distinguished by molecular analysis of PrP^{res}, which involves treatment with proteinase K followed by western blotting of the

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Status	
Extrinsic origin (acquired from another species)	
Human TSE (hypothesis 1) was transmitted to cattle in feed (hypothesis 2) containing raw materials imported from the Indian subcontinent (hypothesis 3)	This potential route of exposure, via imports of mammalian by-products contaminated with human remains for fertiliser and feed during the 1960s–70s, is described in the present paper. Experimental transmission of human TSE to cattle has not been attempted. Strain comparisons show a range of similarities and differences. We argue that this is the most plausible of current hypotheses, but more evidence is needed.
Sheep scrapie transmitted to cattle via feed	Exposure to scrapie occurred for decades, but there has been no satisfactory explanation for why scrapie had never transmitted to cattle before. The theory has become increasingly unlikely with the evidence that (a) cattle are not susceptible to scrapie via the oral route; and (b) all of the scrapie strains studied show substantial differences from BSE, including the fact that scrapie is believed not to transmit to man
TSE in another species (eg, farm, zoo, game, or wild animal) from within the UK or from abroad transmitted to cattle via feed	There is no good evidence to support these theories, as no TSEs in these animals have been reported that could potentially have entered cattle feeds at an appropriate interval before the first known case of BSE in 1985.
Iatrogenic: contaminated medication transmitting TSE from another animal (including human)	An iatrogenic route of transmission was considered an unlikely cause of the BSE epidemic. Although few systematic data were ever presented, no correlation was found between the occurrence of BSE and postulated routes of transmission, including the use of bovine pituitary extract at the time of parturition, or the use of vaccines prepared from bovine materials. ¹⁶
Intrinsic origin (spontaneous event within one or more individual animals)	
PRNP gene mutation; post-translational conversion of PrP ^{sc} to PrP ^{sc}	Sporadic and genetically determined TSEs are known in other species, but have never been identified in cattle. Time course of BSE cases in several countries suggests an acquired mechanism.
Predisposing factors (factors that could predispose to an extrinsic or intrinsic mechanism)	
Host factors	Variation in susceptibility is likely, but no good evidence for specific genetic, immune,* or other host factors has yet been obtained. Although the absence of an immune response to prion accumulation is notable, the lymphoreticular system is involved in the transport of prion protein to the nervous system, and immune factors might play a part in determining susceptibility.
Organophosphate pesticide exposure; high manganese or low copper levels in soil, <i>Acinetobacter</i> bacteria in cattle feed	There is very little experimental or epidemiological evidence that supports these factors. We do not believe that there is a credible case for a primarily autoimmune or bacterial cause for BSE or other TSEs, but have included these hypotheses under factors which might predispose to the development of prion disease.

Table: Theories of the origin of BSE

resulting fragments. More than 270 scrapie cases archived before, during, and since the BSE epidemic have been tested using one of these methods, but no naturally occurring British scrapie strain that bears the characteristics of BSE (webappendix) has been identified. One experimental scrapie isolate, CH1641, has a western blot pattern similar to that of BSE, but differs in its properties on transmission to mice.¹¹ If scrapie and BSE strains were closely related, one would expect both to be transmissible to a similar range of species. However, there is no evidence that scrapie has transmitted to man despite ongoing exposure to infectivity, unlike BSE.

It is notable that all published attempts to transmit scrapie experimentally to cattle by the oral route have failed.^{2,12} Although UK and US scrapie strains have been transmitted to cattle intracerebrally, the clinical course and neuropathology of the resulting illness in the cattle differed markedly from those of BSE (webappendix).¹³ Finally, intracerebral inoculation of natural scrapie into mice transgenic for bovine PRNP produced neuropathological changes that were markedly different from those following transmission of BSE and vCJD, and incubation periods tended to be shorter.¹⁴

Derivatives from other species such as horses, pets, farm, zoo and game animals, and wild animals including unusual species imported from abroad, could in principle have entered animal feed in the UK. Specific proposals that a naturally occurring TSE in wild animals such as Bovidae, Felidae,⁶ or African antelope² could have caused BSE have not been supported by evidence either of the occurrence of such TSEs at the relevant times and places, nor of incorporation of material from these species into cattle feed.

A causative event could have been facilitated by environmental or biological factors including organophosphate pesticides, high manganese or low copper levels in soil, and autoimmune factors (table). However, there is little experimental or epidemiological evidence to support these conditions as causative factors per se.²

The existing theories of the origin of BSE all have significant weaknesses. We propose a new theory, that human TSE-contaminated material was the cause of BSE (hypothesis 1); that this was transmitted orally via animal feed (hypothesis 2); and that the infective material originated in the Indian subcontinent (hypothesis 3). As with any theory of an extrinsic cause, the evidence for potential exposure and for strain compatibility needs to be considered (panel).

The human prion disease hypothesis

Potential exposure

In the 1960s and 1970s, the UK imported hundreds of thousands of tons of whole bones, crushed bones, and carcass parts containing soft tissue of mammalian origin¹⁵ to be used for fertiliser and for the manufacture of animal feed. Nearly 50% of these imports were from Bangladesh (until 1972 known as East Pakistan), India, or Pakistan. Imported materials were sometimes sold in their crude state for use as fertiliser, a largely unregulated industry. Material for production of animal feed was supposed to be sterilised and might be subjected to further rendering. However, feed and fertiliser were often prepared by the same company, or by farmers themselves, and it became apparent to the authorities that material intended for use in fertiliser was being incorporated into animal feed.¹⁶ The poor

controls governing the imports, and the lack of regulation within the countries of origin, particularly those outside Europe, were a source of concern in the UK in the 1960s because of the risk of anthrax and foot and mouth disease.¹⁶ There was no knowledge of any potential risk of transmitting prion disease.

In India and Pakistan, gathering large bones and carcasses from the land and from rivers has long been an important local trade for peasants.^{17–19} Collectors encounter considerable quantities of human as well as animal remains as a result of religious customs. Hindus believe that it is essential for their remains after death to be disposed of in a river, preferably the Ganges.²⁰ The ideal is for the body to be burned, but most people cannot afford enough wood for full cremation, and simply smoking the pelvis in women or the thorax in men has symbolic importance. Many complete corpses are thrown into the river.^{19,20} The practice occurs on a huge scale. In the holy city of Varanasi on the Ganges, some 40 000 funeral ceremonies take place each year at two main sites in the city.²¹ In 2004, a group of volunteers campaigning to reduce pollution retrieved 60 human corpses in 2 days from a 10-km stretch of the Ganges.²²

The inclusion of human remains in material delivered to processing mills has been clearly described.¹⁹ It is highly likely that the incorporation of human remains into exported materials has occurred at least since the late 1950s and may still be continuing. Media reports exist from various countries of trade in human remains, including an account of the prosecution in 2001 of a dealer in Calcutta (on the Ganges delta) for exporting human bones to other parts of India, Pakistan, and the USA.²³

In the late 1960s, during an investigation of anthrax cases in dock workers in the French and Belgian ports on the English Channel, a port medical officer confirmed reports of human material in cargoes of mammalian by-products from the Indian subcontinent.^{24,25} Following the publicity, an animal feed manufacturing company in the UK that made special use of a high-protein meal imported from the region became alarmed, and took the events seriously enough to re-organise its manufacturing facility.²⁶

Why should BSE have started in the UK rather than elsewhere? Several factors might be pertinent. First, the UK was the main recipient of animal by-products exported from India and Pakistan during the relevant period. For example, of Indian exports between 1967 and 1969, the UK received two to six times the amount of any other destination country. Second, Britain was an international leader in research and in commercialisation of increasing milk production by feeding high protein meal to cattle. New sources of imports were actively sought, which would have increased the risk of human-TSE-contaminated meal being fed to cattle, and greater use of meat and bonemeal derived from rendering UK bovine remains increased the likelihood of onward transmission from any primary cases infected by imports.

Panel: Categories of evidence for extrinsic theories of BSE

1 Potential exposure

- 1.1 Environmental route and geography of exposure
- 1.2 Timing
- 1.3 Portal of entry and exposure dose

2 Comparison of disease and strain characteristics*

- 2.1 Comparison of TSE in putative primary species with BSE in field cattle—ie, direct comparison of the diseases as far as possible in their “natural” state
- 2.2 Experimental transmission from putative primary species to cattle
- 2.3 Reverse transmission of cattle BSE to putative primary species
- 2.4 Experimental transmission of putative source, and of BSE, to a tertiary species: mice transgenic for bovine PRNP
- 2.5 Experimental transmission of putative source, and of BSE, to a tertiary species: other mouse lines or other species

*The disease and strain characteristics that may be important for comparisons between the two animals include: incubation period; duration of illness; symptoms and signs; neuropathology; molecular analysis. The route of infection and dose can affect clinical features and neuropathology.

Third, the UK was also a leader in the practice of feeding meat and bonemeal to 1–2-week-old calves,² which might have increased the likelihood of initial cases arising in the UK as well as contributing to propagation of the epidemic. Fourth, changes in rendering practices in the UK have been proposed as a factor facilitating the transmission of TSE infectivity, although experimental data suggest that the effect was small.¹⁶

Little is known about the incidence and types of CJD in the Indian subcontinent. The first case of CJD reported in India was in 1965.²⁷ Between 1968 and 1997, the Indian National CJD Registry recorded only 69 cases.²⁸ However, in developing countries, underestimation inevitably occurs because of limited awareness of diagnostic features, shortage of investigatory facilities, a low autopsy rate, and a low reporting rate even when the disease is suspected.^{28,29} The combined incidence of sporadic CJD (sCJD) and familial CJD (fCJD) in India can be roughly estimated from data established in countries where thorough studies have been done. Assuming that the age-specific incidence of CJD in India is similar to that in these countries,³⁰ we estimate that 150 cases per year would have occurred in India in the late 1960s to 1970s. 80% of the Indian population are Hindus, leading to an estimate of about 120 Hindu people dying from CJD per year. A substantial proportion of the corpses would have been disposed of in rivers, particularly the Ganges, in the traditional manner.

What is the infectivity of a cadaver from a patient with CJD? There are no data about transmission of human infectivity to cattle, but it was possible to transmit human infection by intracerebral inoculation of human brain tissue into primates with as little as 10⁻⁸ g of infective material.³¹ Compared with intracerebral inoculation, the oral route usually requires higher doses to achieve transmission, although exceptions occur.⁵ A conservative

estimate is that a single infected human cadaver could contain about 300 times the ID_{50} for cattle, although we accept the limitations of this type of extrapolation.³²

Prions have legendary resistance to a range of processes that inactivate other types of agent³³ as well as to natural decay.³⁴ None of the natural processes to which a human cadaver may be subjected, nor partial cremation, would be expected to lead to a substantial reduction of prion infectivity. It was established during studies of the BSE epidemic in the 1980s that bovine prion infectivity could survive the whole chain of processes leading to the production of animal feed, including rendering,¹⁰ and the same reasoning applies to the persistence of human infectivity.

Strain compatibility

What type of human prion disease could have been the original cause of BSE? sCJD, fCJD, iatrogenic CJD (iCJD), and kuru existed before the BSE epidemic and are therefore candidates. The original source would be expected to show strain similarities to BSE, and to vCJD, which according to our hypothesis has arisen from reverse transmission. However, strain characteristics can change on transmission between species⁸ and on serial passage in the same species, so one would not expect to find an exact match of all characteristics. The available data are summarised in the webappendix.

Although emphasis has been placed on the differences between the human TSEs (particularly between vCJD and sCJD), there are many overlapping clinical and neuropathological features.³⁵ In fCJD, including Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia, there is wide variation in clinical features, not only between but also within genotypes, many of which overlap with vCJD. The clinical features of all forms of CJD can be affected by polymorphisms at codon 129 of the PRNP gene, and this introduces further variation in the spectrum of clinical phenotypes. There is wide variation among the human TSEs in the local patterns, and distribution within the brain, of the key neuropathological findings of neuronal loss, gliosis, spongiform change and PrP deposition. So-called “florid plaques” of PrP staining were regarded as characteristic of vCJD but have been observed in iCJD and several animal prion diseases, and do not occur in BSE.

Conventional strain typing in a small number of cases of sporadic CJD showed marked differences from BSE and vCJD.³⁶ However, transmission of Gerstmann-Sträussler-Scheinker syndrome to mice³⁷ produced incubation periods close to those of vCJD,³⁶ although different mouse lines were used for the different experiments. Results of molecular analysis of human and bovine tissue samples show that BSE-associated PrP^{res} shares its glycoform ratio with some types of fCJD (E200K and D178N), and has a low Mr-ngc (molecular mass of non-glycosylated component produced following partial digestion by proteinase K) similar to that in many

cases of sCJD, kuru, and iCJD.³⁸ It shares both these parameters with a subtype of fCJD (E200K-129V),³⁹ which although rare could be a candidate for the origin of BSE.

Is vCJD the only phenotype that can result from transmission of BSE to man? Four lines of evidence suggest otherwise. First, every patient with clinical vCJD so far has been methionine homozygous at codon 129 (MM). However, one neurologically asymptomatic patient, heterozygous at codon 129, who had received a blood transfusion from a vCJD patient, was found after death from a ruptured aortic aneurysm to have evidence of PrP^{res} in lymphoid tissue.⁴⁰ Future non-MM clinical cases might occur and could have a different phenotype. Second, experiments in transgenic mice show that transmission of BSE can produce a molecular analysis type typical of sporadic CJD.⁴¹ Third, the incidence of CJD of the sporadic type showed an abrupt two-fold increase in Switzerland in 2001, raising the possibility that the excess cases represented another human disease phenotype resulting from transmission of a bovine TSE to man.⁴² Fourth, the discovery of bovine TSE strain variation⁸ broadens the range of possible similarities between human and bovine TSEs.

Discussion

We have presented substantial circumstantial evidence that human material was imported into the UK with other animal remains used in the production of animal feed over a long period. The incidence of CJD indicates that, on a stochastic basis, infected cadavers would be amongst these remains from time to time. If cattle are susceptible to human TSE transmission by the oral route, it is plausible that these events could have transmitted disease to one or more cattle. Comparisons of human TSE and BSE strain characteristics show sufficient similarities to be consistent with our hypothesis, although the methodology is complex and data are limited.

From the earliest days of BSE, the possibilities of transmission from scrapie or of an intrinsic cause have been recognised. We argue that the paucity of supporting evidence, despite ongoing attention, makes both these theories unlikely (table), and other possibilities need to be considered. We do not claim that our theory is proved, but it unquestionably warrants further investigation (webpanel).

Our first hypothesis, that BSE was acquired from a human TSE, is the most important. No attempts to transmit human TSEs to cattle have been made, and such experiments should be a priority for further research (webpanel). Further work is also needed on transmissions to mice that are transgenic for bovine PRNP, but there are too many uncertainties with such models to obviate the need for direct experimental work in cattle. The second hypothesis, that the route of transmission was oral via cattle feed, is not novel. However, it has proved very difficult to obtain detailed information about sources of

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raw materials and past feed-manufacturing practices; further investigation is still needed. Our third hypothesis, that the origin was the Indian subcontinent, is supported by several lines of evidence, but this and other possible geographical sources should also be investigated further. More research is needed into the types of human TSE occurring in India, other parts of the far east, and any other areas from which human remains may have been imported. An important question is whether some countries are still receiving imports of animal by-products contaminated with human remains. Some of these imports might be used for manufacture of animal feed, thus representing a potential route for new human-to-bovine transmission, and providing a possible explanation for the emergence of new strains of bovine TSE.

Both exporting and importing countries are likely to be sensitive to the implications of our hypotheses, and may feel pressurised to issue denials without adequate investigation. Within as well as between countries, it will be particularly important to establish cooperation between public health, agricultural, and industry organisations, as well as researchers, to try to ensure that further investigations are sufficiently thorough. WHO might be the best international body to coordinate this collaboration.

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