BSE AND CJD UPDATE

- *Fate of the BSE epidemic in cattle*
- *CJD in people*
- *Latest science and policy implications*

Since a new form of human CJD emerged with its postulated link with BSE, the pace of scientific enquiry has intensified - both in terms of understanding the passage of BSE into and between cattle, and in assessing the risks of BSE being transmitted to people. As a consequence, some uncertainties have narrowed.

This note reviews the current state of knowledge on both BSE transmission in cattle and the occurrence of new variant CJD in humans.

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TECHNICAL REPORT
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POSTreports are intended to give Members an overview of issues arising from science and technology. Members can obtain further details from the PARLIAMENTARY OFFICE OF SCIENCE AND TECHNOLOGY (extension 2840).

BACKGROUND

An earlier POST report[1] described what was known of the origin of BSE in cattle, and the basis for concern that 'new variant' forms of a human transmissible spongiform encaphalopathy (TSE) - Creutzfeldt-Jacob Disease (CJD) - had a BSE origin. A second report[2] reviewed mathematical models which had helped understand the spread of BSE in cattle and predict what would happen to the future course of the disease under different cull policies. These reports provided a detailed background on the nature of BSE, CJD and other TSEs, what was known of the methods of transmission and the infectious agent, and the risk to humans from exposure to BSE. To recap briefly:

- the TSEs are characterised by a long incubation period following infection, but an inevitably fatal neuro-degeneration in the brain due to deposits of insoluble proteins and associated holes (which give it the characteristic 'spongy' appearance). The key change in the brain is the transformation of a protein naturally present in the nervous system to an abnormal form.
- The abnormal protein itself appears to be the infectious agent - so ingestion or injection of this so-called 'prion' may transmit the disease. Whether the disease is transmitted and the length of the incubation period, depend on many variables - the dose, the site of administration (across the gut is inefficient, direct injection into the brain very efficient), whether the prion comes from the same or a different species, etc.
- The rapid spread of BSE among cattle was due to infected animals being rendered down and included in meat and bone meal, which was then fed to other animals as a protein supplement. This was stopped in 1988, but contamination may have stayed at a lower level in feed for some subsequent years. The parts of cattle in which most prions would be located were removed from the human food chain in 1989, with the specified offals ban. Even though some doubt remains over the effectiveness of enforcement until the mid 1990s, the main exposure of people to BSE prion will have been prior to the 1989 offals ban.
- Following the 1989 Southwood report, a CJD surveillance centre was established to monitor the incidence of the disease. In 1995 this started to detect 'unusual' cases of CJD, which differed from the previous iatrogenic, sporadic and inherited forms in the age of onset, symptoms and patterns of lesions in the brain. In March 1996, the Spongiform Encaphalopathy Advisory Committee (SEAC) advised that the most likely explanation of these (then 10) cases was exposure to BSE before the introduction of the offals ban in 1989.

The previous POST report had looked at the many data gaps and uncertainties which stood on the way of a proper assessment of the risk to humans of BSE, but research since March 1996 has shed light on a number of these areas, as outlined below[3].

BSE IN CATTLE

The Epidemic

Since 1996, mathematical modelling techniques developed for the AIDS epidemic have been applied at the University of Oxford (in collaboration with the Central Veterinary Laboratory) to explain the progress of the BSE epidemic in cattle, and to infer the relative importance of maternal transmission and feed contamination in the epidemic's continuation. Since the first results were published, the model has been exposed to considerable scrutiny and challenge but after a year in which much further work has been carried out, the original findings have proved to be very robust. Briefly, this model suggests that by the end of 1996:

- Whereas **diagnosed** cases of BSE numbered near 170,000 (and had affected ~60% of dairy and ~16% of suckler herds), nearly 1 million cattle had been **infected** since 1986. Because most were slaughtered before developing clinical signs, some 800,000 infected animals will have entered the food chain; some 446,000 of those before the 1989 offals ban.

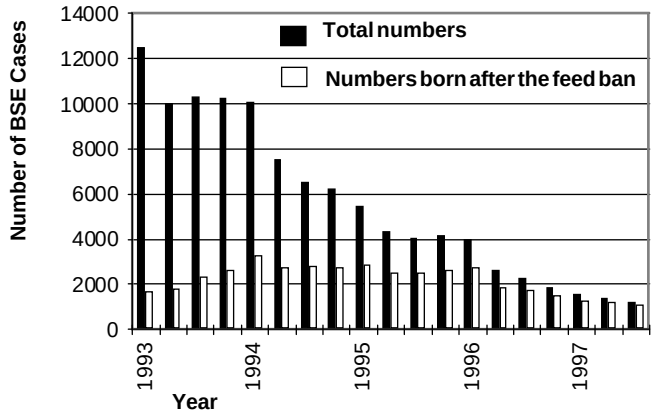
1. **POST report No 78 (April 1996).**
2. **POST report No 85 (October 1996).**
3. **See, for example, the Royal Society 'Update on BSE', 17 July 1997.**

- The increasing proportion of BSE cases in animals born after the 1988 feed ban confirmed continued sources of infection - either continued contaminated feed, maternal transmission, or both.
- The results of the CVL maternal transmission study had indicated that up to 10% of calves born to dams within 6 months of BSE clinical onset went on to develop BSE. The risks of maternal transmission reduces until it is zero for calves born to cows 2 years or more away from exhibiting BSE.
- Initial measures to prevent contaminated feed had been only partially effective - whether because old contaminated stocks had been used, or cross-contamination occurred between ruminant and non-ruminant feed in the manufacturing plant or at the farm. Contamination could only be ruled out finally as a source of fresh infection after August 1996, when it became a statutory offence to possess feed containing mammalian protein on a farm where any livestock were kept.
- The modelling suggests that if maternal transmission is assumed at the rates above, cases of fresh infection from contaminated feed were very few after 1994, since most can be accounted for through the maternal route.
- Now that feed has finally been removed as a source of new infection, the low rates of maternal transmission are insufficient to sustain the epidemic and it has entered a phase of rapid decline (**Figure 1**). If no culling took place and the epidemic were allowed to die away of its own accord, a further 7,000 cases of BSE would be expected between 1997 and 2001. Nearly all of these would be animals already infected, and new infections (essentially all from maternal transmission) have already probably fallen to or below 100 this year.

The 'over thirty months' (OTM) policy was introduced in April 1996[4], and followed by the **selective cull scheme**, adopted as one of the five pre-conditions for the lifting of the export ban agreed at the EC Summit in Florence in June 1996. The Scheme as adopted involved the identification and slaughter of cattle born into the same herd of birth (natal herd) as a BSE case, and which were believed to have eaten the same infected feed as in its first six months of life. Such cattle born between 15 October 1990 and 30 June 1993 would be slaughtered compulsorily, and between July 1989 and 14 October 1990 voluntarily (as there was no legal requirement to have birth records before October 1990). The Oxford model predicted that this agreement would require 120,000 cattle to be culled, with the result that some 1,580 fewer cases of BSE would emerge, up to the year 2001. Around 80 cows uninfected by BSE would be culled for every one infected.

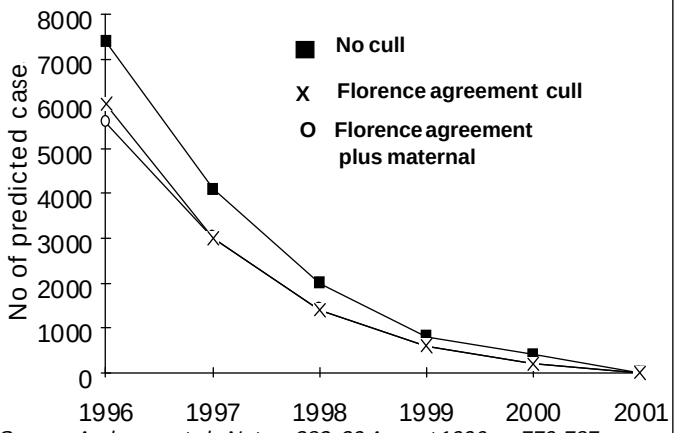
4. The OTM policy requires that all animals bred for human consumption should be slaughtered up to and including 30 months; animals slaughtered after this will not enter the food chain.

Figure 1 BSE CASES: 1993 TO DATE (Case reports)



N.B. Not all cases reported and placed under restriction prove to have BSE (80% were confirmed with BSE in 1996)

Figure 2 FUTURE CASES OF BSE UNDER CULL SCENARIOS



Source: Anderson et al., Nature 382, 29 August 1996, pp779-787

When the Oxford model results became available it was clear that the option agreed at Florence was not the most cost-effective means of reducing the future numbers of BSE cases. There is a high degree of clustering of cases in a relatively small number of herds, so the efficiency of a cull policy depends very much on how effectively such 'high-risk' animals are targeted. The Florence agreement involves some targeting by applying the cull to animals which have come from a herd in which a case of BSE had been reported, and which had been born during the same period (i.e. 1989-93). This should have the effect (Figure 2) of speeding up the fall in BSE cases and 'save' 1,580 of the 6,950 cases predicted to occur from 1997 to 2001. However, concentrating more on high-risk herds and on animals whose mother contracted BSE (see POSTnote 85) would save more cases of BSE (2007) while slaughtering fewer cattle (94,000). Other options could achieve the same reduction in BSE, while slaughtering far fewer cattle, or achieve much greater reductions in BSE by slaughtering the same number of cattle. Although the previous government suspended the cull while it considered the implications of the 'new science' (including the evidence on maternal transmission) ultimately the Florence formula was adopted and is being implemented.

Box 1 IMPLEMENTING THE CULL

MAFF started with a list of farms where BSE cases had been confirmed (or cases had originated from) which fell within the relevant cohort years. The first series of practical steps in implementing the cull is when the veterinary officer (VO) from the State Veterinary Service visits each natal herd of the BSE case. Here the VO has to establish whether the BSE case remained on the natal herd long enough to have received solid feed there. If this is the case, the VO will go on to establish, through the detailed examination of the herd's basic management, feeding, breeding and movement records:-

- the identity of all cattle born in the same calving period;
- if there is no break in calving, those animals born 6 months either side of the BSE case (or, if there is more than one BSE case, 6 months before the first to 6 months after the last);
- what feed was used in this period and when.

In this way, VOs identify which animals are likely to have been exposed to the same risk of infection from solid feed as the BSE case, during the first six months of life. A group of such animals is known as a 'cohort'.

Those cohort animals which have remained in the natal herd are placed under restrictions and the farmer notified of their proposed slaughter. The farmer has the right to appeal and after any such appeal is resolved, the animal is valued and slaughtered. At the natal herd visit, the VO records details of the destinations of cohort animals which have been sold out of the natal herd and these are traced. Once traced they are subject to the same procedure described above.

This tracing of cohort animals represents one of the main difficulties in implementation, as they may be sold on several times. Such tracing can be very time-consuming and also prone to difficulties in view of the possibility of lost tags and the different identification systems used by farmers.

With the 1989/90 cohort, there are two reasons for giving it a lower priority - firstly, these animals would now be 7/8 years old and past the age when they would be most likely to develop BSE, and secondly because accurate records of birth dates did not have to be kept at the time. Moreover, the voluntary nature of the scheme means that a proportion of farmers will decline to have the animal killed even after it has been traced.

In the event, the primary basis of the cull programme has been a mix of science, politics and the practicalities of what could and could not be implemented. The resulting policy targets animals at higher risk of contracting BSE in the 3 compulsory year cohorts of 1990/1, 1991/2 and 1992/3 and (on a voluntary basis) 1989/90. As described in **Box 1**, the animals targeted under the cull are those which have been born into the same natal herd as the BSE case, and are believed to have eaten the same solid feed as the BSE case in the first six months of life. By mid-September 1997, some 30,000 cattle had been culled[6]. The most recent estimate of the number of animals to be culled was 100,000, but this is a maximum figure as it assumes, for example, that there will be full uptake of animals in the voluntary cohort year. It is thus by no means certain that 70,000 animals remain to be tracked down and culled.

6. Expenditure on compensation and disposal amounted to £20 M by the end of July.

As is clear from Figure 2, eliminating the long 'tail' of an epidemic can be difficult - the potentially long incubation period (up to the natural life span of the cow) means that cases of BSE will continue to occur even after all new cases of infection have ceased. Scattered cases may still be present well after 2000, and possibly as late as 2010 in older dairy cows. **The persistence of the long tail of the epidemic is a major impediment to wider acceptance of the extent to which the BSE epidemic in cattle has declined.** While it is not possible to establish whether **any** new infections are still occurring, the Oxford model does place an upper figure of around 100 this year (on the basis of the higher estimate of maternal transmission risk). The same model estimates that only some 300 animals under 30 months old remain which are infected across the whole country.

Research underlines the importance of detailed planning in the cull if the maximum impact on future BSE cases is to be achieved. Even within the constraints of current policy, the order of the cull (focusing on the animals at highest risk first) offers a means of speeding the rate at which the epidemic dies away. For this reason, the compulsory cohort years were dealt with in order, with farm visits beginning with the first compulsory year - 1990-91. A faster rate of decline could also result if animals whose mother developed BSE were also culled. At present, a decision has yet to be taken on whether to include a cull on the animals at risk through maternal transmission (and if so on the selection criteria). The Oxford model suggests that adding maternal targeting to the year cull would reduce future BSE cases (1999-2002) by 5-600 (Figure 2), though this could involve the slaughter of up to 25,000 extra animals.

The final question relevant to the future of the disease is whether there is a possibility of horizontal transmission - as is known to occur with sheep and scrapie. There is a higher chance of cows developing BSE from larger herds, but CVL data can generally relate such trends to feed variations, leaving no residual effects to be explained by horizontal (cow to cow or pasture to cow) transmission[7].

The Origin of BSE

Further light has also been shed recently on the exact origin of the BSE epidemic in cattle. While the 'recycling' of infected animals into animal feed via the rendering process is accepted to be the route of dissemination, there were always questions over the exact nature and origin of the prion involved. The original hypothesis was that sheep scrapie had survived ren-

7. For instance, larger farms will tend to order larger batches of feed, and thus would have had a higher chance of purchasing a contaminated batch. With the passage of time, however, the chances of smaller herds being exposed via feed increases, and indeed there has been a gradual levelling off of incidence relative to herd size.

dering in greater quantities following process changes in the 1980s and had crossed the species barrier and infected cows. The second main hypothesis was that a rare form of BSE had always existed in cattle, or that a rare spontaneous SE, had occurred in a cow which was rendered down, contaminating meat and bonemeal, and spreading the infection to other cattle[8]. Whichever route provided the source, it triggered a rapidly escalating cycle of infection, disease and re-infection.

Some evidence points away from the common form of sheep scrapie being the source:

- Two US studies where scrapie brain has been injected into cows to produce a SE disease did not show all the characteristic hallmarks that distinguish BSE from other forms of SE. This is not definitive, however, since US breeds of sheep and scrapie strains may not be representative of circumstances in the UK. Tests in the UK are planned by MAFF.
- More recent analysis of the epidemiological spread of the disease by the Oxford Group seems to be compatible with (though not proof of) a single point source.
- Against the scrapie origin is the lack of any parallel epidemic in other countries with scrapie and similar rendering processes into animal feed.

The Royal Society now leans to the explanation that the origin of the epidemic could well have been the chance occurrence of a rare spontaneous case of BSE in a cow, or a spontaneous BSE-like case in a sheep. Once such a case had occured however, the key factor was the availability of a route (via the rendering process) through which it could be transmitted to many other animals.

A cattle origin for BSE would raise the possibility that BSE could transmit to sheep to give a different SE than scrapie, which could present a greater hazard to man than scrapie itself. BSE injected into sheep brains does lead to a disease distinct from scrapie, and one of six sheep fed BSE-contaminated cattle brains developed a form of BSE-like disease distinct from scrapie. In sheep, however, 'BSE' develops in the same organs as scrapie, so that there is a risk that the processes which allow horizontal transmission of scrapie might also apply to BSE-infected sheep.

There is no evidence that sheep have actually caught BSE from feed, but the lack of a swift and cost-effective screening test rules out any attempt to look at large numbers of sheep. While this remains a theoretical possibility, there are health implications which are addressed in next section.

8. A third possibility raised by some is that the prion originated in imports of meat and bone meal during the 1970s, although this appears unlikely given that the amounts of meat and bone meal were quite small.

RISKS TO HUMAN HEALTH

The origin of new variant CJD

Since the possible link between cases of new variant CJD and exposure to BSE was first raised by SEAC in 1996, a number of studies have strengthened the scientific case for of such a link:

- Further study has failed to identify any other risk factors which would provide an alternative explanation of the new cases.
- All the cases share the same defining characteristics (including the finding that all victims share the same genetic characteristic of being homozygous for methionine on codon 129 of their PrP gene[9]).
- The detailed structure of the prions in nv CJD can be distinguished from those occurring in sporadic and iatrogenic CJD, and are similar to the pattern observed when animals are infected with BSE (**Box 2**).
- BSE has been transmitted to Macaque monkeys and produces neuro-pathology very similar to nvCJD in humans (and distinct from scrapie).
- BSE prions can be shown to convert human brain protein *in vitro* (as can sheep scrapie prion), although the efficiency of this process is much less than with CJD itself, confirming the presence of a barrier to cross-species infection.
- Work at the BBSRC / MRC Neuropathogenesis Unit at the Institute for Animal Health at Edinburgh has tested brain samples from CJD victims against a panel of different strains of mice which can distinguish between BSE and other SEs on the basis of their incubation time, brain histology and other characteristics (see Bruce et al., Nature, 389, 2 Oct. 1997, 498-501). This work has recently revealed that the outcome of these tests for new variant CJD tissue is indistinguishable from BSE-infected tissue, but clearly different from sporadic CJD tissue, **This suggests that the nvCJD is caused by the same agent as BSE**.
- An identical conclusion was reached by work using mice genetically modified to express the human form of the prion protein. The pattern of infection and disease also distinguished between nvCJD and the sporadic and iatrogenic forms of CJD, and by most of the criteria used, it was BSE and nvCJD which were highly similar (see Hill et al., Nature 389, 2 Oct. 1997, 448-9).

The sum effect of this evidence is that there is now strong and conclusive scientific evidence that nvCJD is BSE in humans[10], but still huge uncertainty over the doses required, incubation period and individual susceptibility. We are thus unable to assess whether the

9. As described in Report no 78, there are three common variants at this position in the population. 40% have the methionine/methionene version; 50% the methionine/valine one and 10% the valine/valine variant.

current cases reflect the few who were exposed to very high doses or were in some way especially susceptible (in which case total numbers of cases may remain few) or are merely the first to succumb to a disease with a long incubation period (in which case numbers would be expected to grow).

So far (October 1997) 21 cases of 'definite and probable' nvCJD have been established in the UK (as well as one in France). Referrals in 1997 of all suspected cases of CJD are running at approximately the same rate as in 1996 (86 up to end August in 1997 compared with 131 in 1996 and 86 in 1995). On past experience however, referrals are a poor guide to diagnostic outcome, and typically half the cases turn out not to be CJD at all.

Forecasting the Future Course of the Epidemic

With additional nvCJD cases continuing to emerge, it is clearly important to assemble a model of the disease's possible future progress as soon as possible. Unfortunately, it is not currently possible to make reliable forward projections because of the lack of knowledge of the incubation period, the dose required for infection and many other aspects (see Postnote 78). For instance, while it is clear that the incubation period for the majority of current cases of nvCJD cannot be much longer than ten years, there is no information on whether this is the mean incubation period or merely the point at which the most susceptible individuals start to succumb. In this context, Kuru is believed to have an incubation period as long as thirty years, and therefore provides no reassurance that the incubation period for nvCJD would be short. Moreover, there may well be other individual or environmental factors still not well understood.

The first attempts to model the disease's progression have been made (**Box 3**), but demonstrate that such calculations are not able to provide any usable forecasts because the uncertainties involved merely place the final scale of the epidemic between fewer than 100 cases and tens of thousands over the next 20 years, and even wider ranges can be produced under other scenarios and assumptions. It may be another 3-4 years before a significantly more reliable picture emerges, making forward planning very difficult. If there is a serious prospect of thousands of cases, urgent research involving the pharmaceutical industry would be required now to have any chance of delivering a treatment in time for use with the rising cases in 5-10 years time. While there is an equally likely prospect of the epidemic being small, however, it remains very difficult to

10. Recent press reports have raised the question of whether the TSEs could be an auto-immune disease, but these reveal a misunderstanding of the science involved. The diseases are quite different and assertions that immuno-deficient mice cannot be infected are simply wrong.

Box 2 DIFFERENT 'GLYCOFORMS' OF THE TSEs

The TSEs result from a process new to medical science where a disease is caused by a naturally-occurring protein which changes shape. The infectious abnormal form, once in contact with the normal form, seems to catalyse a conformational change, even though the chemical composition of the protein (and the DNA sequence in the genes which govern its production in the cell) remain unchanged.

This explanation has always had a difficulty, however, in explaining why there are several clearly identifiable strains of agent in many TSEs, each of which is characterised by different incubation periods, and manifestations of the disease. What seems to be happening is that the relevant proteins may have several possible shapes, and each of these can trigger a change in the normal form into that of the particular infectious prion. The different strains of disease are thus in fact, the result of infection with different shapes of infectious agent.

The different structures adopted by the protein affect the extent to which it can be attacked by enzymes - in some configurations, 'weak' points are exposed; in others, they are hidden. This offers a way of differentiating between the different forms, and recent work at St Mary's Hospital Medical School has shown that a range of TSEs can be classified into 4 types, according to the way they respond to enzyme attack, as shown below:

RESPONSE OF DIFFERENT TSEs TO ENZYME ATTACK

Prion Type	Exhibited by % of cases
1	sporadic CJD (20) iatrogenic CJD (15)
2	sporadic CJD (80) iatrogenic (15)
3	iatrogenic (70)
4	nvCJD (100): BSE-infected animals (100)

According to these tests, all 10 cases of new variant CJD exhibit type 4 behaviour, which is clearly distinguishable from the other types of CJD - sporadic (types 1 and 2) and iatrogenic (mostly type 3). What is particularly significant is that the brains of BSE-infected cattle, cats, kudu and macaques all showed a type 4 pattern, suggesting strongly that new variant CJD and BSE share these common features, which are distinct from other types of CJD.

Not only does this strengthen the likelihood of a direct link between BSE and nvCJD, but this 'glycoform signature' also offers a means of distinguishing different strains of TSE much more readily than is currently possible. For instance, this test might be deployed to check whether sheep have become infected with BSE (as distinct from scrapie); also the possibility that infection might be detected from samples of tonsils or lymph node biopsy, thereby avoiding the need for brain biopsy in suggested nvCJD cases.

More recent work by the Edinburgh Neuropathogenesis Unit has applied the technique to different scrapie strains and has shown that some strains may be differentiated by this method, but that others do 'overlap' with the different types 1-4 for BSE and CJD. Further work to increase the resolution of the technique is thus required before it can provide a 'fingerprint' for all TSEs.

Sources:

Collinge, J., et al., 1996. "Molecular analysis of prion strain variation and the aetiology of new variant CJD." Nature 383, 24 October, pp 685-690.

Aguzzi, A. and Weissmann, C., 1996. "A suspicious signature". Ibid, 666-667.

Somerville, R.A., et al., 1997 "Biochemical Typing of Scrapie Strains". Nature 386, 1 May, 564.

Box 3 MODELLING FUTURE CASES OF CJD

Assuming that new variant CJD is due to exposure to the BSE agent, it is possible to use mathematical models which simulate the development of epidemics to predict the future course of CJD, if the following are known:-

- the incubation period,
- how the population was exposed to the BSE agent over time.

A first attempt at this was made recently by a team from the London School of Hygiene and Tropical Medicine, in collaboration with the National CJD surveillance unit, based on the cases of CJD with clinical onset in 1994 and 1995. In view of the small number of cases (13) in this period the model can predict very different outcomes depending on the assumptions put in.

A key assumption is the incubation period, and the estimated number of infections is extremely sensitive to this. The shortest likely incubation period is 10 years; the mean incubation period for CJD caused by contaminated human pituitary hormone is currently around 13 years; but the incubation period for kuru can be as long as 30 years. The longer the incubation period, the more likely it is that the human epidemic may be large (table below). The wide range within each incubation period depends on the assumptions made on the effectiveness of the offal ban and other factors influencing human exposure.

RANGE OF POSSIBLE FUTURE HUMAN CASES (next 20 years)

Mean Incubation Period	Range of Possible Future Cases
10	75-213
15	107-1595
20	162-12,000
25	245-80,000

Clearly, with such wide ranges, the current epidemiological models are of no use as a precise predictive tool, or for planning a public health response. However, as additional cases arise, each year's results will allow re-calculation, and should give considerable narrowing in the range of future predictions.

Source: Cousens, S.N., et al., 1997. "Predicting the CJD epidemic in humans" Nature 385 16 January 1997, pp 197-198.

justify major R&D investments in searching for such a treatment. Recently the DH has set up a SEAC subgroup to look at the cases of nvCJD and epidemiological models to identify any emerging trends as soon as possible.

Prospects for Early Detection

The unusual nature of the infective agent makes its detection very difficult. Since the 'rogue' protein is the same basic chemical structure as the normal one and no genetic material has been found to be involved, there are no simple tests for its presence in feed, in cattle, or in humans. Even now, the only way of diagnosing an affected animal (or person in the case of CJD) is by the symptoms of the disease, confirmed by examining the brain after death. Any test for the infectious prion itself must detect the shape, not the composition of the PrP, or it must discover some second-order but specific consequence of the abnormal protein's presence (e.g. a different metabolite), or symptoms such as early neurophysiological changes resulting form the disease. A diagnostic test to detect traces of the infectious prion before the onset of symptoms, whether in cattle or in people, remains a very high research priority.

The earlier POST report pointed out that attempts to find some 'tell-tale' in urine, cerebro-spinal fluid (CSF) or blood are underway. The CVL has patented a urine test which may aid diagnosis of BSE at the end phase of the disease, though its use as a predictive tool has yet to be validated. Recent work in the USA, UK and France focuses on 'indicator' proteins in the CSF which are a by-product of the brain damage associated with the disease. For instance, the CVL work has shown that apolipoprotein E is elevated in CSF in BSE-affected cattle but not normal cattle, and could be detected at late stage incubation. The proteins are released when the nerve cells in the brain degenerate, so are unlikely to offer a means of screening for cattle with sub-clinical BSE. Progress is however, also being reported in making immunological tests sensitive enough to the prion in biopsy samples to provide a possible confirmatory test that an animal is BSE-free, provided that the ideal tissue for biopsy sampling can be identified. Unfortunately, tonsil and lymph node, which are of use in sheep and possibly humans, do not have detectable levels of prion protein in cattle.

Research at St Mary's Hospital has also found that the prion may be present in tonsils before the onset of clinical symptoms, and a tonsil biopsy has been used in one recent case to confirm a provisional diagnosis of nvCJD, using the 'glycoform' signature test (Box 2). Despite these leads, a more sensitive diagnostic test for infectious prion remains a high research target.

Potential Treatments

Another consequence of a prion origin is that many 'traditional' cures will not work, since the infective agent is not recognised as foreign by the body's immune system, and is not vulnerable to antibiotics, antivirals etc. Thus, none of the SEs exhibit any inflammatory response around the lesions, or any sign of the prion having triggered an immune response and being attacked by lymphocytes. Nevertheless, research at the University of Zurich shows that the immune system must be involved in some way, because mice which have been genetically engineered to lack an effective immune system can only be infected by injection directly to the brain, and not through peripheral tissue. This suggests that the immune system may be involved in some way in transporting the prion from the initial point of infection, and suggests that the transport of

prions from gut to brain is a far more complicated process than simply their absorption from the gut, and gradual spread via lymph or blood throughout the body. The process seems to be that the normal proteins in the peripheral tissues must first be progressively converted to prion, from whence it can spread to the spinal cord and the brain. This insight does offer a possible line of research into means of interrupting or slowing the spread of the agent from the initial site of infection.

Another finding is that the incubation period depends on how active the individual's gene is in expressing the prion protein. If a pharmacological method could be developed for reducing the expression of the protein, this might thus reduce the rate of spread. An additional obvious approach is to find some drug which somehow interrupts the chemical conversions of the prion protein from the normal form into the infectious form. However, although this process has been shown to occur in the laboratory and can be observed[11], it is not yet actually understood.

Prion Infected Material and the Food Chain

Historical. The MRC Biostatistics Unit examined the results of two surveys of British Adults' diets during 1986/7 (when the levels of infectivity in beef products may have been rising) and in 1992/3 by which time the offals ban should have reduced infectivity substantially. Both surveys showed a striking gradient in the percentage who ate burgers and kebabs - from 45% in the 18-24 age bracket to only 13% in the 50-64 age group. Such findings, partial though they are would help explain the numbers of young people in the nv CJD cases. But a clearer picture of the fate of potential infective offals during the 1980s will emerge from a study funded by MAFF (at Leatherhead Food Research Association) which is attempting to provide a broad overview of the fate of bovine tissues between 1980 and 1995 and identify categories of tissues and by-products appearing to pose the greatest risk in the final products consumed. Particularly important will be to track the fate of spinal cord and mechanically-recovered meat prior to the effective operation of the offals ban, and to indicate what types of food are most likely to have contained them.

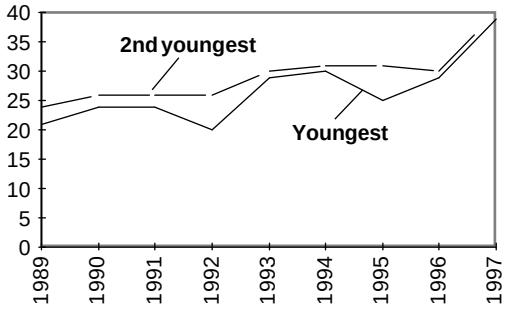
Present day. Since late 1989, the exclusion of offals should have removed most infectious material from the human food chain, although doubts as to the adequacy of separation and hygiene practices in some abbatoirs up to 1996 led to major reorganisation and redirection within the Meat Hygiene Service to improve the effectiveness of enforcement. The original measures have been strengthened by prohibiting additional organs thought to be capable of harbouring the infectious prions, and further safety margins have been introduced with the OTM policy since April 1996 (**Box 4**). There remain no indications that there is any infectivity in striated muscle or milk.

Box 4 THE OVER THIRTY MONTHS POLICY

The OTM policy originated from a recommendation in April 1996 from SEAC that any cattle aged over 30 months should be deboned under supervision in special plants to ensure that all potentially infectious material was removed carefully. In the event the practicality of these proposed arrangements and consumer resistance to buying older beef led to the overall OTM scheme whereby the sale of all beef from cattle over 30 months old is prohibited. The scheme was subsequently reflected in EU regulation 716/96 of 19 April 1996. The scientific rationale behind 30 months was that this comprised half the average incubation period, the point at which research on scrapie in sheep and mice suggested that infectivity entered the central nervous system. Given that the evidence so far suggests that the brain and spinal cord represent the greatest weight of infectivity in infected cattle, very few cows under the age of thirty months would therefore contain significant levels.

As the feed ban became more effective, the dose received by those animals which became infected should have fallen, which would lenghen the incubation period. Indeed, the age of youngest onset of BSE is increasing (see Figure) and this year it is 39 months so far. These factors have led some to call for the 30 month scheme to be made a 36 month scheme which could help some forms of beef production while not significantly affecting the levels of safety from a human health viewpoint.

This however, would require a more reliable documentation of age to replace the current one based on teeth emergence. Moreover, to be absolutely sure, August 1996 could be taken as the point beyond which no significant exposure took place, so that 30 monthly beyond this,the 'OTM' rule could be progressively rolled back.



Current concerns focus on whether there are any residual (albeit low-level) routes through which possible infectious material might enter the human food chain. One concern is that cases of BSE in other countries are going unrecognised which could provide a source through imports of meat which has been prepared in slaughterhouses not applying the same standards for separation of offals.

11. Trials in mice have shown that some agents (e.g. strong antifungals, congo red dye) can delay infection. Growing PrP in cell culture also offers a means of screening for active substance, and some (e.g. pentosan or heparin sulphates) can inhibit the prion conversion *in vitro*.

Recent work by vets from the UK, Netherlands and Germany predicted how many animals exported from the UK to European countries would have been expected to succumb to BSE if they followed the same pattern of infection as those remaining in the UK. The estimates in **Table 1** are subject to a number of caveats, but they do suggest that the scale of BSE recorded officially in other EU states is an underestimate. The potential consequences of this are that:

- there is a possibility of infected animals entering the food chain - hence the proposal, agreed at the July Agriculture Council Meeting, that all abattoirs should separate the bovine offals from human consumption, as has been done in the UK since 1989.
- the existence of indigenous BSE means that local sources of MBM may be contaminated. While MBM containing ruminant protein was prohibited across the EU as feed for ruminants in 1994, there is still scope for cross-contamination, accidental feeding of MBM for pigs/chickens to cattle, and the other factors which caused the epidemic to become so prolonged in the UK.

As discussed earlier, there is the theoretical possibility that BSE could transmit to sheep to give a SE which is more infectious to humans than scrapie[12]. The only way of differentiating between scrapie and BSE at present is to take brain samples and submit them to expensive and very time-consuming experiments on panels of different strains of mice. This could not provide the basis of a screening programme to see if any sheep has been infected with BSE in practice, and more work needs to be done to develop the molecular biological approach where the different types of prion are differentiated (see earlier). Since it will clearly be impossible to prove that BSE has not infected some sheep, consideration has been given to the precautionary measures of removing potentially affected parts from the food chain. Legislation introduced in September 1996 already prohibits human consumption of the heads of sheep and goats, but other potentially infectious sheep offal is still entering the food chain - parts of the gut used to make sausage skins, spleen and lymph nodes still used in various meat preparations, and spinal cord which is difficult to remove from sheep. In this context, SEAC has recommended that, until it is established that there is no BSE-like spongiform encephalopathy in sheep, it would be a sensible precaution to eliminate head, spinal cord and spleen from the human food chain. The Government is currently consulting on implementing this, though the removal of spinal cord is not practical in animals below one year old.

Table 1 NUMBERS OF CASES OF BSE PREDICTED IN CATTLE EXPORTED FROM THE UK

Country	*Number if imports were:*		*Number*
	Beef only	*Dairy only*	*reported*
Greece	0	0	-
Belgium/Luxembourg	2	24	-
Denmark	3	40	1
France	4	43	27
Netherlands	5	62	2
Italy	6	68	-
Spain	6	74	-
Germany	30	334	5
Portugal	26	370	72
Ireland	103	1263	234

Source: Schreuder, B.E.C., et al., 1997. "Risk of BSE from the import of cattle from the UK into countries of the EU". Vet.Rec., Aug.23, 187-190.

RESEARCH AND IMPENDING RESULTS

A substantial research programme continues funded primarily by MAFF, DH and BBSRC (although others such as the Wellcome Trust play an important role in certain critical areas). The list of projects is long and too detailed for this overview, but an important development in 1996 was for the DH Director of R&D to define a strategy for research into the human health aspects of TSEs. This emerged in November 1996, and identified priorities in the following areas:

- the natures of the prions involved and whether more than one strain of nvCJD is involved;
- determining the relationship between BSE and CJD and how it is transmitted;
- developing efficient diagnostics;
- public health implications (e.g. for food and environmental safety, safety of medical procedures and decontamination methods);
- develop therapeutics for CJD;
- surveillance and epidemiology - to check whether cases are being overlooked (e.g. in the older population) and to develop predictive models for CJD in the population.

The strategy on human health aspects of TSEs will be followed by another, addressing animal health aspects of TSEs, in the near future. Since it will inevitably have much in common with the one produced by the DH it is intended that they will eventually be merged. Although much work is already under way, the strategy will identify further areas of research that are needed. Others will inevitably be identified as interim or final results of existing research are published in the coming months - for instance, MAFF is embarking on an examination of tissues from sheep and goats for signs of scrapie, and this will also evaluate whether any cases could have a BSE rather than scrapie origin.

12. There is no evidence that scrapie has passed to humans at any stage in the last 200 years.