Official statements and other publications; however without the evidence about BSE and „MBM infectiosity“

Contents

Introduction

The Blair government has warned that vCJD, caused by eating beef infected with „mad cow disease“ (BSE) could claim many as 250,000 lives. This is double the previous estimate of 136,000 possible deaths and means that the government is now working on a „worst case scenario“ of one in every 250 people in Britain dying from the disease. Putting the risk into context, microbiologist and leading CJD expert Dr Stephen Dealler said on average people in the UK had eaten 50 meals made from the tissue of an infected animal.“At the moment the number of cases of CJD we are seeing are doubling every year. (www.wsws.org/articles/2000/nov2000/bse-n03.shtml). If the double for a long time then the numbers are in millions, if they double for just a few years then the numbers are in thousands….(HYLAND, 2000).

On the other hand, ADAMS (1990) researcher from Kings College (London) reported that current concerns about the cattle disease BSE do not appear to take cognisance of the parallel with scrapie, the similar/ identical disease of sheep/ goats. This has existed for 200 years, and clearly involved a longstanding consumption of meat/offal from affected animals, but apparently without consequential human disease. A summary is given of the characteristics of the human and animal spongiform encephalopathy diseases and their causative agents. The conclusion of the official Southwood report that the bovine disease is caused by the inclusion of sheep scrapie material in cattle fodder is critically discussed; an alternative mechanism is proposed for its emergence (ADAMS, 1990).

Similarly see 15 years later, an international study of the epidemiologic characteristics of CJD that was established in 1993 and included national registries in France, Germany, Italy, the Nedherlands, Slovakia, and the United Kingdom (UK). In 1997, the study was extended to Australia, Austria, Canada, Spain, and Switzerland. LADOGANA et al (2005) pooled data from all participating countries for the years 1993 to 2002 and included deaths from definite

or probable CJD of all etiologic subtypes. **This study has established overall epidemiologic characteristics for CJD of all types in a multinational population- based study**. They found; four thousands four hundred forty-one cases were available for analysis and **included 3,720 cases of sporadic CJD, 455 genetic cases, 138 iatrogenic cases, and 128 variant cases**. The overall annual mortality rate between 1999 and 2002 was 1.67 per million for all cases and 1.39 per million for sporadic CJD. Mortality rates were similar in all countries. There was heterogeneity in the distribution of cases by etiologic subtype with an excess of genetic cases in Italy and Slovakia, of iatrogenic cases in France and the UK, and of variant CJD in the UK. They concluded, that **intercountry comparisons did not suggest any relative change in the characteristics of sporadic CJD in the UK, and the evidence in this study does not suggest the occurrence of a novel form of human BSE infection other than variant CJD**. However, this remains a possibility, and countries currently unaffected by variant CJD may yet have cases (LADOGANA et al. 2005). This study has established overall epidemiologic characteristics for Creutzfeldt–Jakob disease (CJD) of all types in a multinational population–based study. Intercountry comparisons did not suggest any relative change in the characteristics of sporadic CJD in the United Kingdom, and the evidence in this study does not suggest the occurrence of a novel form of human bovine spongiform encephalopathy infection other than variant CJD. However, this remains a possibility, and countries currently unaffected by variant CJD may yet have cases (LADOGANA et al., 2005).

Dietary data suggest that people in Britain in the 1980´s and 1990´s have had widespread exposure to the BSE agent through consumption of burgers, pies and other products containing mechanically recovered meat as spinal cords and paraspinal ganglia were not prohibited from inclusion in such meat until December 1995. Yet, in spite of this widespread and lengthy exposure, only 108 definite and 42 probable cases (without neuropathological confirmation) of vCJD have been diagnosed over 11 years in a population of 55 million. In addition, only two cases of vCJD were observed in 2005.

Scientific proof that BSE causes vCJD rests upon the fact that BSE was first diagnosed 10 years before vCJD was diagnosed. Therefore, the temporal relationship between BSE and vCJD only coincidentally supports the notion that BSE caused vCJD, and as such is not strong evidence.

BSE is claimed to have occured as a result of a subtype of scrapie infecting cattle through ingestion of contaminated offal. However, *t*here has been no demonstration that feeding cattle meet and bone meal produced from sheep has led to the development of BSE since introduction of the MBM is fact; the assumption that the fall in incidence of BSE is due to this ban is no more than an assumption… (BROWN, 2001).

In other words, it is the refusal of the medical officers to admit that their first assumptions about BSE have been shown to have been wrong by subsequent findings which has enabled the media and the politicians to produce this completely unfounded scare. In the late eighties the public were told that BSE could not be transmitted to humans. Then in the mid 1990s they were told that it had „crossed the species barrier“ as though acting in the manner of an invading army?

So, despite continued investigation the origin of BSE is not certain - there is no proof of how long subclinical BSE and vCJD existed in the UK before this time. See the official statements or „the position views“ of DEFRA-United Kingdom (2004-2005) , IFST- United Kingdom (2004), FAO- ROME- Dr. John W.WILLESMITH (1998) about the BSE, during continued investigation…; and in addition the „BSE opposite theory“ from BATH UNIVERSITY- Dr.David R.BROWN (2003 and 2001).

1.Position of DEFRA (Department for Environment Food and Rural Affairs); about the BSE
a/ Pathogenesis, Epoidemiology, Diagnosis, Transmission of BSE (2005-2004)

(http://www.defra.gov.uk/animalh/bse/science-research/diagnos.html)

PATHOGENESIS; 25 October 2005

Pathogenesis refers to the study of the disease process; the sequence in which the tissue of the body became infected and the progression of clinical signs both in experimentally and naturally infected animals.

Primarily, undertanding the disease process provides information on which, and at what time during infection, tissues of a BSE infected animal may be infectious to other animals. This is important both for animal and public health.

This area of research is intended to:

- determine the distribution and mechanism of spread of the agent in the body
- identify and characterise the causative agent.
- identify host factors that influence susceptibility to the disease.
- compare the biology of BSE with that of other TSE's

Causative agents of TSEs

There is still considerable scientific uncertainty about the precise nature of the causative agents of TSEs. The prion protein PrP is a normal membrane-associated protein that is found most commonly in the central nervous system and is very important in the development of TSEs. Modified forms of this protein are associated with infectivity and also accumulate in the brain in the diseased state. The function of the unmodified prion has not yet definitively established.

Put simply, the **prion** hypothesis says that infectivity is caused by a structurally-modified form of the PrP that promotes conversion of other PrP molecules into the same form. These then accumulate to interfere with the function of nerve cells. However, whether the prion (the PrP protein alone, with no associated nucleic acid) is the cause of BSE is not certain.

Other theories suggest that the causative agent might be a "**virino**"; an infectious pathogen containing a core of nucleic acid associated with host derived cellular proteins, similar to a small virus. Alternatively, some scientists have argued that a **filamentous virus** is the cause of TSE.

The prion hypothesis may be the most popular at present, but a number of scientists find difficulties with it, primarily as it contradicts the scientific orthodoxy that the inheritance of a trait, such as disease, must be associated with nucleic acid. MAFF holds no particular position with respect to the nature of the agent as all hypotheses currently remain unproven. This does not, however, mean that all novel hypotheses will be tested by research as most can be tested for a fit with current scientific knowledge about BSE.

This scientific uncertainty over the nature of the causative agent does not, however, affect the validity of the steps taken to control the disease and protect human health. The basic tenets on which the controls were based, namely, that the infective agent is transmitted through

contaminated feed and detected only in certain tissues, even in clinically affected animals, apply, whatever the actual form of the agent.

Research on pathogenesis

Three of the main experiments carried out under this heading are described below. They all involve bioassays, where groups of animals are inoculated to assay for infectivity. Ideally, results of such experiments would not be put in the public domain until the work is completed to ensure that all the information derived from them is available for interpretation. However, a continuous review of data is also carried out to ensure that the most significant findings can be used in policy decisions as soon as possible.

Distribution of Infectivity

In this study a group of calves was challenged (infected) by mouth with 100g of homogenised brain from confirmed cases of BSE. At the time that the experiment was started the amount of brain needed to infect a calf was not known. In this experiment a 100g dose was intended to guarantee infection. In actuality, this dose is probably 10 -100 times greater than most cattle will have been exposed to via feed.

Calves were challenged simultaneously, and then slaughtered at set time intervals. A total of more than 40 tissues/fluids were collected for testing for infectivity. The aim was to trace the routes by which infectivity spread through the body of the calf from infection to eventual onset of clinical disease. Various methods of tissue examination (histopathology, SAF detection and immunohistochemistry) have been used to monitor the movement of infectivity.

The last tissues were taken at 40 months after infection, when the cattle displayed clinical symptoms. At least 3 years were required for completion of the testing of the tissues for infectivity. Currently the only available technique to detect infection is by testing them in mice. Although any infectious tissues should give results in fewer than three years, to reliably confirm a result as negative can potentially take the full lifespan of the mice.

Up 18 months post infection, the only evidence of infectivity has been found in the wall of the lower small intestine (distal ileum). This was an expected result because of the presence of Peyer's patches (lymphoid tissue) in the wall of the intestine, which are thought to be responsible for distribution of the agent around the body. As infectivity was detectable as early as six months post-infection the definition of specified bovine offals was extended to include the intestines of calves.

Infectivity was detected in the intestine, the brain, spinal cord, dorsal root and the trigeminal ganglia of calves killed more than 18 months post infection. Bone marrow was slightly infectious when clinically affected.

Clinical signs were first seen at 35 months post infection in this experiment compared with a mean of 60 months in naturally infected cattle. This reflected the high infective dose used. The application of all available diagnostic tests to the brains of animals in this experiment failed to find consistent changes prior to three months before the onset of clinical signs.

References

Jones V. et al. 1996. Protein markers in cerebrospinal fluid from BSE-affected cattle. Veterinary Record. **139**. 360-363.

Wells, G.A.H. et al. 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. Veterinary Record. **148**, 5, p103-106

Infectious Dose Experiment

This experiment was designed to determine the minimum dose that would cause BSE infection in a cow. It should be noted, however, that in the epidemic itself, cattle did not receive raw brain. Brain would have been heat treated during rendering and subsequently diluted with other feed constituents.

Calves were challenged by mouth with homogenised brain from confirmed cases of BSE. Some received 300g (3 doses of 100g), some 100g, 10g or 1g. They were then left to develop

BSE, but were not subjected to the normal stresses that they might have encountered in a dairy herd. Animals in all four groups developed BSE. There has been a considerable spread of incubation period in some of the groups, but it appears as if those in the 1 and 10g challenge groups most closely fit the picture of incubation periods seen in the epidemic. Experiments in progress indicate that oral infection can occur in some animals with doses as low as 0.01g and 0.001g.

Sensitivity of methods to detect BSE

The use of mice to test bovine tissues for BSE infectivity has come under criticism by some researchers. Inevitably, in transmitting from one species to another there is some loss of sensitivity. The so-called 'species barrier' has to be crossed.

When the early assays for BSE were established there was no other means of testing for the presence of infectivity in tissues, short of infecting cattle. However, mouse models used for detecting scrapie were known to detect infectivity in a range of peripheral tissues where one might expect a lower concentration of the agent. There was therefore no reason to suspect that they would be any less sensitive to BSE. In fact, it seems that BSE from cattle brain affects mice more frequently and uniformly than does any known strain of scrapie.

Nevertheless, it did prove necessary to test the extent to which mice were less sensitive to BSE than cattle. Serial dilutions of brain homogenate from BSE cattle were used to inoculate both cattle and mice directly into the brain. Mice were found to be around 500 times less sensitive to BSE than cattle.

Tissues negative in mouse bioassay (from the pathogenesis experiment) are being checked in cattle bioassay to ensure that low levels of infectivity are not missed. Such experiments are expensive and lengthy because of the long incubation periods involved. It is hoped that future transgenic mice, carrying bovine genes will improve the sensitivity of mouse assays sufficiently to limit the need to use cattle.

Transmission has not occurred in cattle or mice inoculated with either spleen or lymph node pools derived from BSE cattle. This is in direct contrast to the results from sheep with natural scrapie infection.

EPIDEMIOLOGY of BSE; 21 October 2005

Early studies

In response to the emergence of BSE, epidemiological studies were started in April 1987. The initial objective was to obtain detailed data from a study of 200 cases. These investigations provided interpretable results at the end of 1987.

The study looked at a wide range of different factors which might have been responsible for BSE or could have affected the occurrence of the disease. These included herd type, size and age structure; whether the animals had been in contact with; whether animals were homebred, bought into the farm, or imported; the origin of breeding animals and use of artificial insemination etc.; details of pharmaceutical products, vaccines, pesticides and herbicides used on animals and on the farm; and details of feeding practices.

Research findings

This initial epidemiological analysis showed the pattern of BSE cases to be typical of an extended common source epidemic (i.e. an epidemic involving many individual, independent disease outbreaks, which can each be traced back to a common source). No evidence was found of cattle to cattle transmission, nor of any common exposure to specific pharmaceutical products or pesticides (explicitly including organophosphorous compounds). Nor was there any evidence that the disease was simply genetic. The possible direct transmission of the scrapie agent from sheep to cattle, by either direct or indirect contact, was an untenable hypothesis as there were no sheep present on around 20% of the farms affected with BSE.

The only common feature of all the cases of BSE investigated was the use of commercially produced compound feed containing meat and bone meal (Wilesmith, Wells, Cranwell & Ryan 1988 Vet. Rec. 123, 638-644). This conclusion was further supported by the fact that the incidence of BSE in dairy herds was much greater than in beef suckler herds, closely matching the use of compound feeds in these two types of herd. Subsequently a formal case-control study of calf feeding practices and meat and bone meal inclusion in proprietary concentrates provided substantiating evidence (Wilesmith, Ryan &Hueston 1991 Res Vet Sci 52 325-331)

Initial causes of BSE

Epidemiological analysis suggest that the primary cause of BSE is consumption of contaminated feed. However, if this was the case, it is unclear what the initial source of the BSE agent in feed was. There are four main hypotheses (listed below) (Refs: Kimberlin 1996, Kimberlin and Wilesmith 1994, Wilesmith et al 1991) and in each case changes to rendering procedures in the 1970s/1980s (Wilesmith, Ryan and Atkinson 1991, Vet Rec 128, 203) would have allowed the infectious agent to survive during rendering of animal by-products into meat and bone meal (MBM) and so enter cattle feed.

(i) **Origin from scrapie in sheep**; This hypothesis is possible as scrapie has been prevalent in the British sheep populations for at least two centuries and there has been a significant increase in the sheep population in Great Britain from 1980 onwards, and possibly a resulting increase in the prevalence of scrapie. This explanation probably best fits the epidemiological data.

(ii) **Exposure to rare sporadic BSE**. It is possible that BSE is a naturally occurring and long established disease of cattle, but which occurs extremely rarely (rather like sporadic CJD in humans). Passing infectious material from a BSE-infected animal through a rendering process no longer capable of destroying the agent could have led to contamination of the cattle feed chain. However, there is no reason why, if BSE is a natural, sporadic but exceedingly rare disease of cattle, that it should be confined to the UK, but no traces of the disease have been diagnosed before 1985 in other countries. Similarly, the occurrence of BSE in other countries is consistent with exposure from imported cattle or meat and bonemeal from Great Britain. This explanation, though consistent with the epidemiological data, seems less likely than a scrapie origin.

(iii) **A new strain of scrapie which was particularly infectious to cattle** might have arisen and then entered the cattle feed chain through meat and bone meal. One would expect any such new strain of scrapie to emerge first in a single flock of sheep and so any epidemic resulting from exposure to it to begin in a geographically localised area. However, the BSE epidemiology shows a geographically widespread occurrence with simultaneous onset and not one which starts from a single point of infection, and this explanation is not consistent with the epidemiological data.

(iv) **Imported African bonemeal** - Early epidemiological investigations ruled out the use of imported feed ingredients as a factor in the epidemic. Several cases of spongiform encephalopathy have been diagnosed in captive ruminants in British zoological collections. Some have been proved to be transmissible, and in particular TSE disease in kudu and BSE appear to be identical. While the most likely explanation is that the exotic ruminants became infected by the same route as British cattle, via concentrate feed containing meat and bone meal, it has also been postulated that the BSE epidemic may have arisen from an African source

This is pure speculation based on the diagnoses in the exotic ruminants and the fact that small consignments of "bone meal" were imported into Britain in the 1970s. It is almost impossible to identify specific consignments so many years in arrears, and their size suggests that the meal was used for horticultural fertiliser rather than as feed ingredient.

However, Botswana was identified as a particular source of the "imported meal", but the structure of the cattle industry in Botswana, the exclusion of wild life from the abattoirs that would have produced the rendered bone meal (or meat and bone meal), coupled with the fact that TSEs have not been diagnosed in cattle or wildlife in Botswana or Africa suggest that the hypothesis is tenuous. The source abattoirs are EC approved and cattle are subject to veterinary ante- and post-mortem examinations. A most recent theory suggests that the origin of BSE in Britain could be from a single TSE-affected African antelope from a wildlife park in the UK. Such an animal could have been rendered and subsequently fed to other cattle.

Regardless of the initial origin of BSE in cattle, it is clear that the epidemic was sustained and boosted by the recycling of BSE infected cattle material to other cattle from the mid 1980s onwards. The vast majority of cases have therefore been caused by cattle material being fed to other cattle.

Uncertainty of the precise origin of BSE does not affect the control measures taken - the ruminant feed ban removes both sheep and cattle material from ruminant rations, and is designed to prevent transmission in feed between, as well as within, species.

Incidence of BSE

Despite the large numbers of cases confirmed over a period of more than 11 years, at its peak the epidemic only affected approximately one per cent of adult breeding cows per year. The peak was in the winter of 1992/93 when a maximum of just over 1,000 suspected cases a week were being reported. Not all suspects that are placed under restriction are eventually confirmed, the remainder either recovering or being killed and found not to be affected with BSE. The percentage of suspects not confirmed has increased naturally during the epidemic.Since 1992 the incidence has fallen year by year at a rate of around 40% per year, although the decline in 1997 was approximately 55%.

Although there have been some herds that have suffered large numbers of cases, this is unusual. Approximately 74 % have had five cases or less, 35% have had only one but one herd was found to have as many as 124 cases. Most of the herds affected are dairy herds(63%), 27% are beef suckler herds, and the remainder are mixed beef and dairy type. Because BSE is predominantly a disease of dairy cattle, a total of 81% of all cases are of dairy origin. 61% of all dairy herds have had at least one case, whereas only 16% of beef suckler herds have had a case. However, a large number of the beef suckler cases were born and probably infected in dairy herds, and subsequently brought into beef herds.

The picture described above simply reflects the fact that dairy cattle receive far more supplementary feed, which used to contain meat and bone meal, than beef suckler cattle, which are predominantly grass fed.

Horizontal and vertical transmission

Horizontal and vertical transmission refers to the transfer of infection from animal to animal, except that vertical transmission refers specifically to spread from parent to calf. A specific form of vertical transmission is maternal transmission, which refers to spread from cow to calf, either whilst in the womb or afterbirth.

Defra has funded specific studies to attempt to determine whether or not BSE does spread by these routes. However, the fact that the within herd incidence of BSE rose no higher than 2.7% is a good indicator that infection does not spread from cow to cow to any significant extent.

Horizontal transmission

If any transmission does take place at all, the evidence from sheep scrapie suggests that the calving cow probably represents the greatest risk to other cattle. A recent study has shown that lambs born to ewes introduced into a scrapie flock became infected and died at the same age as lambs born to native ewes, consistent with lateral transmission of scrapie to lambs (Research in Veterinary Science 76 (2004) 211–217). While there has been a study showing

some evidence for horizontal transmissions up to three days after calving, there was no evidence of transmission to the cow's own calf, (Hoinville et al. 1995. Veterinary Record. 136, 312-318). However, the results were considered statistically insufficient to suggest that horizontal transmission was occurring.

Another method of testing this theory involved the feeding of calves with placenta from confirmed cases of BSE. The calves that were challenged were clinically normal prior to slaughter and their tissues are being assayed for infectivity to find out if the cattle became infected without succumbing to clinical disease. Some preliminary tissue assays have been completed, without any infectivity being detected.

Professor Anderson's group's work, has given rise to speculation about the possibility that horizontal transmission may arise on farms. The conclusion state quite clearly that:-

"it should be emphasised that to date no evidence exists supporting the hypothesis of direct horizontal transmission." and,

"produces no evidence to support the hypothesis that horizontal transmission is occurring at a rate sufficient to allow BSE to become endemic"

Their, and previous studies have however indicated that bigger herds are at greater risk of acquiring BSE than smaller herds.

This correlation of risk with herd size has in fact been known and published for several years. (***Wilesmith et al, in Veterinary Record, 130. 90-94. Wilesmith, 1996, Bovine Spongiform Encephalopathy. The BSE Dilemma (p45-55).***

The finding is consistent with the original suggested explanation that the larger the herd the greater the probability of purchasing an infected batch of feed.

Vertical transmission- from the sire

Epidemiological studies have compared the incidence of BSE in the offspring of healthy bulls and those of bulls that later were confirmed to have BSE. There was no difference between the groups that could be attributed to the BSE status of the bull. Further data obtained from Artificial Insemination (AI) organisations have been analysed in order to expand and update previous studies, but no risk from semen used for commercial AI has been identified.

Experimental transmissions have also been attempted using semen, seminal vesicles and prostate of bulls confirmed to have BSE, but no infectivity was detected in these samples. (Wilesmith, J. W. 1994. New Zealand Veterinary Journal. **42.** 1-8).

Maternal transmission

In July 1996, the Spongiform Encephalopathy Advisory Committee (SEAC) advised that maternal transmission would not sustain the BSE epidemic in cattle in the UK.

In October 2002, scientists at Imperial College London published a revised epidemiological analysis which estimated the risk of maternal transmission of BSE to be less than or equal to 1% in the last six months of the maternal incubation period.

In 2003, SEAC were advised of the potential future change from culling all offspring born after July 1996, to a cull of offspring born within two years of the clinical onset of disease in the dam. SEAC advised that they saw no scientific grounds for maintaining the policy for culling offspring in the UK in place at that time.

Reference:

Donnelly, C.A et al. (2002) Implications of BSE infection screening data for the scale of the British BSE epidemic and current European levels. P*roc.R.Soc.Lond.* B (2002) **269**, 2179-2190

Continued risks from contaminated feed

The ongoing epidemiological monitoring attempted to establish the reasons for the continuation of the epidemic in animals born after the ruminant feed ban of July 1988. In the absence of maternal and/or horizontal transmission there had to be another reason for the continuation.

Although the ban clearly worked very well, it seems not to have been absolute. In particular there has been a shift of the epidemic after the ban both northwards and eastwards. There was a direct correlation between the incidence of BSE in cattle born in 1990 or later with the size of the local pig and/or poultry population. There was no direct correlation between the presence or absence of these species on farm and the incidence of BSE in cattle on the same farm. The pig and poultry populations are at their densest in the northern and eastern parts of England. Feed manufactured for such farms was usually, but not always, produced in mills that also compounded feed for cattle and other species. It was perfectly legal, until the end of March 1996, to include ruminant protein, in the form of meat and bone meal, in pig and poultry rations. Until September 1990 that meal would also have included meal derived from rendering specified bovine offals. Further investigations led to the conclusion that, feed manufacturers that produced products for cattle as well as pigs and/or poultry ran a risk of accidentally incorporating ruminant protein in their cattle rations.

Additional investigations involving experimental challenge of calves by mouth with brain from confirmed cases of BSE indicate that as little as 1mg of brain can infect calves. This highlights the importance of tight quality control in feed mills in preventing cross contamination on a scale that could still infect cattle. In 1996, a decision was taken to ban the use of meat and bone meal altogether in order to eliminate that risk, after testing of feed produced in mills indicated that it was extremely difficult to prevent cross-contamination.

Predictive modelling

In 1996, Anderson and others published analyses of the epidemic which agreed with Defra's own modelling results (Anderson R. M. et al. 1996. Nature. 382. 779-788 and Anderson R. M. et al. 1997. Nature, 386. 302). These were that the epidemic was in decline, and that if maternal transmission was taking place it was at low frequency. This means that even without further intervention the epidemic could be expected to dwindle to insignificant proportions by the year 2001.

The data recorded on the Defra database has been further analysed with a view to targeting infected cattle on farm, but which are currently healthy, so that they can be slaughtered and destroyed before they actually develop clinical disease. Options for slaughter, described elsewhere under the "Selective cull" rely on the considerable epidemiological analyses that have been carried out on this data over a period of years.

In April 1998 the Cattle Identification Regulations 1998 were put in place. Cattle born after 1/1/98 must have a Defra approved eartag in each ear and a corresponding cattle passport. Cattle Tracing is an integral part of the Government's efforts to improve consumer confidence in beef, and will have the potential to provide researchers with data which could help improve targeting and/or predictive modelling of the BSE epidemic.

A computerised Cattle Tracing System (CTS) was launched in Great Britain on 28 September 1998. The Cattle Tracing System (CTS) is the fourth element in a comprehensive system of cattle identification and registration. Just as changes in ownership of cars are logged from the time the car is first sold to the time it is scrapped, data relating to where cattle are kept is recorded by the Government so that the animals can be traced for a variety of reasons, including animal disease.

The CTS will make it possible to:

- check which animals are present on a holding
- check where an animal has been during its life
- trace animals exposed to a disease risk
- give assurances to buyers about an animal’s life history, and so
- strengthen consumer confidence in beef

Organophosphates (Ops) and BSE

An organic farmer from Somerset, has campaigned against the use of organophosphate chemicals for many years. With respect to BSE he has suggested that the use of organophosphates, and one in particular, phosmet, has been linked to BSE cases in cattle.

In April 1997, he was invited to put his proposal to SEAC at a meeting which was attended by representatives of the Advisory Committee on Pesticides and the Veterinary Products Committee. At the meeting it was agreed that additional data on the topic should be made available to allow him to continue with his investigations, before SEAC could decide whether further research was needed to test his hypothesis.

At their meeting of 24 October 1997, **SEAC considered further data which led them to conclude that OPs did not accumulate in cattle, which they would need to do in order to cause disease.**

SEAC also considered the question of further research into a possible role for OPs and concluded that more evidence would be required to justify further consideration of a role for OPs in the epidemiology of BSE. Proponents of the theory are free to apply to funding agencies for resources to conduct such experiments. However, on the evidence to date the Committee did not feel that special priority should be given to this area of research.

DIAGNOSIS of BSE; 12 July 2004

BSE diagnosis is a key area of research, whose main aims are:

- the improvement of current post-mortem diagnosis and the development of new tests
- the development of BSE-specific diagnostic tests applicable to the live animal both in pre-clinical and clinical phases of infection
- investigation of new approaches to diagnosis.

Aims

Defra started funding research towards developing a diagnostic test early on in the epidemic. The development of a live test is particularly difficult with TSEs because the specific infectious agent is difficult to isolate and there is no detectable immune response - normally two prerequisites for developing diagnostic tests.

The main aims of Defra research into development of a diagnostic test are:

- distinguishing cattle affected with BSE from suspect cattle showing similar clinical signs caused by other conditions;
- identifying infected animals and herds before the onset of clinical disease, thus facilitating surveillance and eradication;
- detecting infection with the BSE agent in carcasses in abattoirs;
- differentiating between BSE and scrapie

Funding

Q. Who funds and carries out research on BSE and CJD?

A. The main Government funders of research on BSE and the other TSEs of animal and public health significance are Defra and the Biotechnology and Biological Sciences Research Council (BBSRC). The Department of Health (DH), the Medical Research Council (MRC) and the Scottish Executive also fund work on CJD and public health aspects of TSEs. Total Government spending on research into BSE, scrapie, CJD, and other TSEs was increased to over £37 million in 2002/03, with around £240 million allocated in total since 1986. Some charities also fund TSE research, notably the Wellcome Trust.

The European Commission has also funded work on TSEs. In December 1996 the Commission developed an Action Plan for TSE research based on a report from Professor Weissmann. As a result of this initiative over ***400 research projects*** have been funded to examine a number of aspects of BSE and other TSEs of significance for animal and public health. Defra provides support to UK researchers in a number of these projects including the supply of tissues and scrapie-free sheep.

Research is carried out in a wide number of leading scientific institutions in the UK, including universities, hospital medical schools, Government Agencies and Institutions.

Q. How much is Defra spending on BSE and scrapie research?

A. Between 1986 and 2003 Defra spent over £100 million on research into BSE and related diseases. The overall research budget for BSE in the current financial year (2003/04) is over £16 million.

By the end of the financial year 2002/03 UK government funders (Defra, BBSRC, MRC and DH) had spent over £240 million on research into TSEs affecting animals and man. This large programme, which has been developed progressively since the beginning of the BSE epidemic in cattle, is designed to provide the scientific basis for policy decisions directed at the eradication of BSE from the national cattle herd and the protection of public and animal health. The research covers, amongst other areas, epidemiology, diagnostic procedures and test development, transmission, pathogenesis of disease and studies on the nature of the causative agent.

Q. What comprises Defra's research programme?

A. Defra research aims to develop our basic knowledge of TSEs and provide important information to guide policy to protect public and animal health. This is a large and detailed programme that has been built up since the discovery of BSE in 1986 and can be divided into four main categories:

- **Epidemiology** - to confirm the origin of the disease, to follow the course of the epidemic and to monitor the effectiveness of statutory control measures;
- **Pathogenesis and the nature of the agent** - to study the mechanisms of the disease and its cause;
- **Diagnosis** - development and validation of methods of BSE diagnosis and detection.
- **Transmission** - to identify routes of transmission of the agent, to investigate the possibility of maternal transmission, to identify which species are susceptible to infection and to determine how the genetic profile effect's susceptibility to infection.

However, these are not rigid classifications and there is considerable overlap in the nature of the studies.

Co-ordination

Q. Who co-ordinates research / funding?

A. Although Defra is the largest funder of TSE research, the FSA, BBSRC, the Wellcome Trust and DH all have significant programmes and carry out important work. There is co-ordination of research activity within Defra and between appropriate departments and other research sponsors through the TSE Joint Funders Group.

In addition, The High Level Committee on Research and Development into TSEs was set up in January 1997, this comprises of senior Government officials and reporting to the Cabinet Office. Its purpose is to ensure progress is made in placing research by Government funders and to resolve any difficulties impeding progress.

Scrapie Research

Q. What research is underway into scrapie & the possibility that BSE can be transmitted to sheep?

A. Studies have shown that sheep can be infected with BSE under experimental conditions by intracerebral injection or feeding brain material from cattle with clinical BSE. This indicates a theoretical possibility that some sheep could have contracted BSE through consumption of contaminated feed before the ban on MBM in animal feed. Defra has a large research effort directed at the study of experimental BSE and scrapie in sheep (see above). A breeding flock of scrapie-free sheep has been established using animals imported from New Zealand where no cases of scrapie have been reported and they will be used extend these and other studies.

Q. How much is being spent on research related to TSEs in sheep?

A. Approximately half of the Defra TSE research budget of £16 million in 2003/04 was allocated to research projects related to scrapie and BSE in sheep. The BBSRC also funds research on scrapie.

Q. How might strain typing work be applied to the question of BSE in sheep?

A. The clinical signs of experimental BSE in sheep cannot be distinguished from those of scrapie. Therefore, if the BSE agent strain is present in the national flock, its occurrence may be masked by scrapie. Currently, there is a concerted effort being directed at developing and validating reliable diagnostic tests that can both detect and differentiate scrapie and BSE infection in sheep. VLA scientists, Professor John Collinge and scientists from the Institute of Animal Health, have all worked on the use of molecular strain typing technology to establish whether the technique will distinguish between scrapie and BSE and if so whether it can be practically applied. As yet definitive answers have not been produced (See Hill *et al*, Neuroscience Letters, Volume 255, 1998 and Hope *et al*, Journal of General Virology, Volume 80, 1999). In sheep, there are confounding factors, such as genetic effects of the host animal and the possibility that the strain type of the agent may have altered after passage within the sheep flock, which complicate strain identification. Further work is in progress to assess whether these molecular techniques can fully differentiate TSE agent strains in sheep.

Q. What techniques are currently being used to look for BSE in sheep?

A. It is commonly accepted that natural scrapie can be caused by several different strains of the agent. However, it is not simple to differentiate which agent strain is present in cases of scrapie. Currently, the only way to define scrapie strains is to infect a panel of genetically defined mice with brain derived from a scrapie affected sheep. Each scrapie strain has a well characterised and reproducible incubation period and brain pathology in the panel of mice. However, this method to characterise the scrapie agent is a lengthy process (up to two years) and expensive.

In his publication in Nature on 24 October 1996 Professor Collinge described new work on strain typing prion protein using a molecular analysis technique rather than bioassay in laboratory mice. He had identified "fingerprints" for new variant CJD, classical CJD and BSE. With this method BSE and new variant CJD appear to have the same fingerprint. The fingerprint is based on differences in physical behaviour of prion protein molecules. His work has been recognised as support for a possible connection between BSE and the new variant CJD. However, subsequent work with scrapie in sheep has shown that the patterns derived from scrapie are more complex, difficult to interpret and may not be able to define scrapie strains as clearly as it appears to with CJD.

BSE and risks to man

Q. Which parts of a cow are infected with BSE?

A. Defra has funded a large experiment to detect infection in a selection of tissues from cattle. The interim results were published in The Veterinary Record 31 January 1998. This large experiment tests an extensive range of tissues for infectivity from cattle experimentally infected with BSE throughout the course of infection. Infectivity, as tested in experimental mice, has been identified in the central nervous system, the peripheral nervous system (dorsal root ganglion - which is close to the spinal cord) and distal ileum (the lower small intestine).

Q. What is the status of diagnostic tests for detecting BSE infection in live cattle

A. Defra is funding several projects that aim to identify and define specific disease markers that could be used to diagnose BSE. These include examining urine and other mediums such as blood and spinal fluid. However, for a test to be effect it must be highly specific for BSE and be sensitive enough to detect levels of infection before the development of clinical disease. This has not yet been accomplished but efforts are continuing to isolate possible markers.

Q. How is work progressing on rapid BSE post-mortem tests for carcases?

A. The EU recently published results of a specifically commission independent evaluation of four rapid post mortem diagnostic tests. The results of this evaluation are promising and the author concluded that some of the tests had 'excellent potential for detecting or confirming clinical BSE for diagnostic purposes. The high specificity indicates that these tests may be useful for general post mortem screening of older animals'. (see Nature,1999, Vol. 400, p105). More information about research into diagnostic tests can be found here.

TRANSMISSION of BSE; 12 JULY 2004

Bioassays for infectivity

Bioassays are the gold standard for detecting the infectivity of tissues. They are therefore used in transmission experiments to provide a model for assessing the likelihood that infective tissues could act as agents for transmitting disease.

A bioassay is able to detect infectivity directly, rather than by relying on a correlation with the presence of modified PrP as detected by immunological or histological methods. The tissue to be tested is either fed to, or injected into the experimental animal, which is then observed for signs of the disease. Based on a combination of observations incubation period and brain pathology, assumptions can be made about the infectivity of the tissue and type of disease. This is called strain typing.

The majority of TSE bioassays are conducted in panels of inbred mice. These are groups of mice where individuals within a group are genetically similar and so exhibit comparable responses to the disease. Bioassays in mice allow a large amount of data to be generated in a relatively short time and reduce the need to experiment on larger mammals, which would be more slower, more expensive, and ethically less acceptable.

The effectiveness of murine bioassay is clearly demonstrated by the success of the assay in detecting BSE infectivity by both intra-cerebral and by oral routes of exposure. Panels of inbred mice have been used for many years for the study of scrapie. BSE transmits more readily from cattle to mice than does scrapie from sheep.

Transgenic mice

In recent years much progress has been made in the production of strains of mice that carry PrP genes from other species, sometimes instead of their own gene, sometimes in addition to their own. Such techniques have been shown to permit infection of mice with certain strains of TSE when previously they may have been resistant. This work has been led by American and Swiss groups but expertise is expanding in several laboratories in Europe.

Techniques such as deletion and substitution of genes, and the production of chimaeric (mixed) genes are helping to obtain a greater understanding of the role of the PrP gene in infection. As a simple assay model it seems possible to reduce incubation periods following inoculation by increasing the number of copies of PrP genes that are carried by the mouse. The difficulty with such multi-copy models is that they are difficult to reproduce consistently, and the gene is usually not in its natural position in the chromosome. Alternative transgenic models, which may behave more naturally, involve the removal of the mouse gene and its replacement by the PrP gene of another species. While mice carrying human or hamster genes have dominated this field so far, projects are under way both in Britain and abroad to

produce mice that carry PrP genes of cattle or sheep. If successful they may lower incubation periods and sensitivities of assay and so permit more rapid progress.
One of the leaders in this field in Britain is Professor John Collinge at the Neurogenetics Unit, Imperial College of Science, Technology and Medicine at St Mary's Hospital Medical School, London. He has published already on transmissions into mice carrying human transgenes. Although the work was primarily targeted at CJD he has also challenged some mice with BSE.
With deletion of the mouse PrP gene, and insertion of the human gene, he has been able to infect mice with CJD and its variants far more successfully than unmodified mice. When such mice were challenged with BSE infected brain they appeared far more resistant than to CJD, and indeed more so than normal non-transgenic mice. Care is needed in interpreting this result as an indication of the degree of susceptibility of humans to BSE infection.
One reason for caution is because the PrP gene sequence can vary, and the mice used in the initial experiments were homozygous in coding for the amino acid valine at codon 129. All the cases of new variant CJD identified so far have been homozygous for methionine at this point, as are 40% of the UK population, and therefore further experiments are needed to test whether the identity of the amino acid at position 129 is critical to rendering humans susceptible to BSE.

Infectivity in tissues

Experiments to determine whether or not infectivity was present in the tissues of clinically affected cattle began in 1987 with the inoculation of mice with brain from field cases of cattle affected with BSE.
By 1988 there was clear evidence that BSE was transmissible to mice via inoculation of brain tissue.
The aim of the tissue assays was to identify which, if any, of the tissues that might be consumed by humans contained detectable quantities of infectivity. This would of course be of significance in determining the pathogenesis of BSE too. A large number of tissues were inoculated into mice, usually by a combination of intracerebral and intraperitoneal routes
The initial assays identified infectivity only in brain, spinal cord and retina of the clinically affected cattle. We are aware of no experiments that have detected BSE infectivity in blood using the mouse bioassay. However,it should be noted that transmission has been observed after blood transfusion between sheep (Hunter N., et al. (2002) Transmission of prion diseases by blood transfusion. J. Gen. Virol. 83; 2897-2905). SEAC opinion on the relevance of this finding has been published here.
Subsequent work investigating distribution of infectivity in cattle has used experimentally infected animals. Assay of cattle in intermediate stages of incubation are vital to our understanding of the extent to which humans have been exposed to BSE. Most beef comes from cattle killed at around 18 months to two years of age, long before onset of clinical disease.
In contrast, infectivity in scrapie infected animals is distributed through many more tissues than BSE in cattle.

Infectivity to other species

Initial attempts to transmit BSE to other species served two purposes. Firstly to identify a suitable laboratory test which would rule out the requirement for cattle bioassays. Secondly, challenges in other species would inform discussion on risks to those and other species during the course of the epidemic.
Most of the species challenged have been challenged either parenterally (usually by intracerebral injection, occasionally accompanied by intravenous and/or intraperitoneal injection) or orally.

Species that have been infected both parenterally (by injection) and orally are: mice, calves, sheep, goats, mink.
Sheep orally challenged with bovine BSE have succumbed to the disease. Results to date demonstrate that infectivity is found distributed similarly to scrapie i.e in both central nervous system and in peripheral tissues.
Transmission studies of BSE to domestic fowl and pigs by injection and oral exposure to BSE brain homogenate.
Chickens and pigs have been exposed to far more contaminated meat and bone meal than have cattle in the past. Meat and bone meal was regularly used in their diets at higher inclusion rates than in ruminant rations. It is often argued that infection in such animals would never be seen because they are slaughtered too young. The adult pig population in the United Kingdom is however large enough for disease to appear and be detected. The clinical symptoms from parenteral infection are similar enough to viral neurological diseases such as Classical Swine fever, which are notifiable. This give confidence that such an infection has not been missed.
Whilst pigs are susceptible to BSE infection if multiply injected with BSE brain homogenate, they have not been shown susceptible to BSE when fed orally. A range of tissues from these orally fed pigs were bioassayed in mice, and none found infective.
Meanwhile, studies on chickens that have been challenged with BSE have shown that chickens do not develop the disease. Some birds from this experiment did show abnormal neurological signs. Nervous system tissue was collected from these birds and inoculated into new birds and mice. Neither the birds nor mice have shown signs of infection to date.

b/ EPIDEMIOLOGY of BSE, DEFRA - BSE information – last updated 30 April 2001 (http://www.defra.gov.uk/animalh/bse/bse-science/level-4-content-epidem.html)

Research findings
The initial epidemiological analysis (Wilesmith et al., Vet.Rec., 1988; 638-644) showed the pattern of BSE to be a typical of an extended common source epidemic (i.e. an epidemic involving many individual, independent disease outbreaks, which can each be traced back to a common source). The possible direct transmission of the scrapie agent from sheep to cattle, by either direct or indirect contact, was an **untenable hypothesis as there were no sheep present on around 20% of the farms affected with the BSE.**
The only common feature of all the cases of BSE investigated was the use of commercially produced compound feed containing meat and bone meal (Wilesmith et al., Vet.Rec., 1988; 638-644). This conclusion was further supported by the fact that the incidence of BSE in dairy herds was much greater than in beef suckler herds, closely matching the use of compound feeds in these two types of herd. Subsequently a formal case- control study of calf feeding practices and meat and bone meal inclusion in proprietary concentrates provided substantiating evidence (Wileesmith et al., Res.Vet.Sci., 1991; 325-331).
Incidence of BSE
Despite the large numbers of cases confirmed over a period of more than 11 years, at its peak the epidemic only affected approximately one per cent of adult breeding cows per year. The peak was in the winter of 1992/93 when a maximum of just over 1,000 suspected cases a week were being reported. Not all suspects that are placed under restriction are eventually confirmed, the remainder either recovering or being killed and found not to be affected with BSE. The percentage of suspects not confirmed has increased naturally during the epidemic.
Since 1992 the incidence has fallen year by year at a rate of around 40% per year, although the decline in 1997 was approximately 55%.

Although there were have been some herds that have suffered large numbers of cases, this is unusual. Approximately 74% have had five cases or less. 35% have had only one. One herd has had as many as 124. Most of the herds (63%) affected are dairy herds, 27% are beef suckler herds , and the balance are of mixed beef and dairy type. Because BSE is predominantely a disease of dairy cattle, a total of 81% of all cases are of dairy origin. Kooking at the national herd, and the proportion of it that has been affected, we see that 61% of all dairy herds have had at least one case. Only 16% of beef suckler herds have had a case, but a large number of these were born and probably infected in dairy herds, and subsequently bought into beef herds.

The picture described above simply reflects the fact that dairy cattle receive far more supplementary feed, which used to contain meat and bone meal, than do beef suckler cattle. They are predominantly grass fed. The fact that the within herd incidence of BSE rose no higher than 2.7% is a good indicator that infection does not spread from cow to cow to any significant extent. The results were considered insufficient to suggest that horizontal transmission was occurring.

HOINVILLE et al.: An investigation of risk factors for cases of BSE born after the introduction of the „feed ban". Vet.Rec., 136, 1995; 312-318

The Anderson conclusions

Professor Anderson´s group´s work, has given rise to speculation about the possibility that horizontal transmission may arise on farms. The conclusion state quite clearly that;-

„it should be emphasised that to date no evidence exists supporting the hypothesis of direct horizontal transmission", and,

„produces no evidence to support the hypothesis that horizontal transmission is occuring at rate sufficient to allow BSE to become endemic".

Their, and previous studies have however indicated that bigger herds are at greater risk of acquiring BSE than are smaller herds, and they suggest that further research is necessary to clarify why rhis might be.

This correlation of risk with heard size has in fact been known and published for several years. Data on geographical risk and variations in risk according to herd size were published Wilesmith et al., in Veterinary record (1992). All the factors mentioned have been monitored and have been incorporated into continuing analyses. The greater risk for large herds was more evident during the early years of the epidemic. **As the epidemic has progressed the average adult herd size of newly affected herds has decreased**. The finding is consistent with the original suggested explanation that the larger herd the greater probability of purchasing an infected betch of feed. However, as time went on the cumulative probability of small herds purchasing an infected batch of feed increased up to the time when ruminant proteins were witdrawn from feed and the average size of BSE affected herds therefore decreased.In addition there is no evidence that **small herds were managed differently, with respect to their feeding regime, from larger herds**. This changing association therefore does not provide any supporting evidence for horizontal transmission. A notable feature of the epidemiology of BSE is that in 50% of affected herds cases of BSE have only occurred in one birth cohort (i.e. born in one year´s calving season) and these animals have not been a source of infection for the rest of the herd. Some large herds fortuitously or actively changed feed sources at critical times, and despite having relatively high incidence of disease in cattle exposed to particular feeds have subsequently experienced no disease in cattle that have been fed from the new supplies. Similarly, **where infected cattle have been purchased (in other words clinically normal but unknowingly infected before purchase) and introduced into farms where the risk of exposure via feed was low (for example in Scotland**), they have not caused infection in subsequent generations of animals.

Predictive modelling.

In 1996, Anderson and others published analyses of the epidemic which agreed with MAFF´s own modelling results. These were that the epidemic was in decline, and that if maternal transmission was taking place it was at low frequency. This means that even without further intervention the epidemic could be expected to dwindle to insignificant proportions by the year 2001…

In April 1998 the Cattle Identification Regulations 1988 were put in place. Cattle born after 1/1/98 must have a MAFF approved eartag in each ear and a corresponding cattle passport. Cattle Tracing is an integral part of the Government´s efforts to improve consumer confidence in beef, and will have the potential to provide researchers with data which could help improve targeting and/or predictive modelling of the BSE epidemic. A computerized Cattle Tracing System (CTS) was launched in Great Britain on 28 September 1998. The CTS is the fourth element in a comprehensive system of cattle identification and registration. Just as changes in ownership of cars are logged from the time the car is first sold to the time it is scrapped, data relating to where cattle are kept is recorded by the Government so that the animals can be traced for a variety of reasons, including animal disease.

References

ANDERSON,R.M. et al.: Transmission dynamics and epidemiology of BSE in British cattle. Nature, 382, 1996; 779-788

WILESMITH,J.W.- RYAN,J.B.M.: Absence of BSE in the offspring of pedigree suckler cows affected by BSE in Great Britain. Vet.Rec., 141, 1997(10); 250-251

WILESMITH,J.W.- WELLS,G.A.H.-RYAN,J.B.M. et al.: A cohort study to examine maternally- associated risk factors for BSE. Vet.Rec., 141, 1997(10); 239-243

WILESMITH,J.W.- WELLS,G.A.H.- HOINVILLE,L.J.- SIMMONS,M.M.: Suspected vertical transmission of BSE. Vet.Rec., 134, 1994(8); 198-199

2. Position of IFST (Institute of Food Science and Technology); about the BSE (2004)

The explanation from The Institute of Food Science and Technology "BSE and vCJD in humans" *(www.ifst.org/hottop5y.htm)* **(Information Statement, October 2004), (http://www.ifst.org/uploadedfiles/cms/store/ATTACHMENTS/BSE.pdf) they say:**

Debate about the origin of BSE in cattle is and will probably remain unresolved, including the respective possible roles of recycling of scrapie infected sheep or possible low-level BSE-infected cattle, of imported MBM, of changes in rendering processes and the use of solvents, of the effect of organophosphates at sub-toxic levels, of the influence of trace metals, of the possible role of exposure to bacteria showing cross-reactivity with nervous tissue, and of hay-mites as possible vectors.

From the outset, successive IFST Position Statements (now called "Information Statements") have pointed out that in real life, simple "single cause -> single effect" relationships are rare, and it seems quite possible, or even probable, that BSE developed as a result of a number of coincidental factors coming into play in a combined circumstance. It is doubtful whether it will ever be possible to prove either the origin of BSE or the precise combination of factors that led to its amplification and infection of cattle.

The Report of the BSE Inquiry concluded BSE probably originated from a novel source early in the 1970s, possibly a cow or other animal that developed disease as a consequence of a gene mutation, rather than rendering of sheep infected with "normal" scrapie. In response, the UK Government asked Professor Horn to lead a small team of scientists to look in greater detail at the origin of BSE by pulling together all scientific understanding, including emerging findings, on the subject. Whilst differing in respect of the scrapie theory, the review team's findings do agree with many of those in the BSE Inquiry Report. Although the Horn et al review at www.defra.gov.uk/animalh/bse/bseorigin.pdf does not come to a firm conclusion about the origin of BSE, it does offer a possible explanation as to why BSE first occurred in the UK. This followed a rare sequence of events between 1970 and the 1980s: meat and bone meal was introduced into **the feed of dairy calves from the first or second week of age - young animals are thought to be more susceptible** to infection; and this, coupled with changes to the rendering process which may have affected the degree of infectivity, could have created a particular set of conditions which enabled the BSE agent to spread. The group also considered a number of specific questions relating to the first appearance of the disease. Of particular note, was their conclusion that although BSE may have originated as a result of a mutation in cattle or sheep, this was no more likely than certain other possibilities. They agreed that it was not possible to exclude an unmodified scrapie agent as the original source of BSE and found the evidence for this theory more persuasive than most.

Other theories considered included one that BSE started in an African ungulate, the carcass of which got into meat and bone meal. The review team did not rule this out entirely, but believed that the theory could not be substantiated. They did not consider organophosphates were the primary source of BSE as the theory was not consistent with the evidence on the spread of the disease. They agreed with the BSE Inquiry that the autoimmune hypothesis was not viable.

Nevertheless, there can be little doubt that the key factor in the subsequent development of the epidemic was the use of MBM as cattle feed, as demonstrated when its prohibition led to successive year-by-year reductions in confirmed new cases, as the following graph shows, falling in Great Britain (from the peak of 36,680 in 1992, to 1,311 in 2000. Note that data for subsequent years (781 in 2001, 445 in 2002, 174 in 2003) are not strictly comparable with earlier data because the large number of UK cattle killed and either buried or incinerated on pyres during the 2001 UK foot and mouth disease outbreak must have included an unknown number incubating BSE*(www.ifst.org/hottop5y.htm)* .

3.Position of FAO (FOOD AND AGRICULTURE ORGANIZATION) of the United Nations; about the BSE (Rome, 1998)

(http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/003/W8656E/W8656E00.HTM)

Manual on Bovine Spongiform Encephalopathy (John W. WILLESMITH)

Creutzfeldt-Jakob disease

Two basic forms of TSE occur in humans. Gerstmann-Strdussler syndrome (GSS) has a genetic basis and manifests as an autosomal dominant disease. It is classically associated with

the codon 102 Pro-Leu change. CJD occurs in a sporadic form, which accounts for 85 percent of cases, and as a familial form. In the latter case there are now known to be several point mutations and expansions in an octapeptide repeat sequence within the PrP gene open reading frame associated with the disease (Poulter *et* al., 1992).

The sporadic disease is very rare and remarkably uniform throughout the world, affecting approximately one in a million people. Since CJD was first known to be transmissible in 1968, the possibility that the disease was associated with scrapie has been studied widely and intensively, but with no supporting evidence.

A new variant of CJD ($_{nv}$CJD) was identified in March 1996 in the United Kingdom. It was classified as a new variant because of differences in the histological changes in the brain, because of clinical signs and because to date (October 1997) it has predominantly affected people less than 40 years of age. So far 22 cases have occurred in the United Kingdom and one case in France, where BSE has also occurred, but at low incidence (see Chapter 4). Its occurrence raised the level of concern that the BSE agent was responsible. The initial results of strain typing of $_{nv}$CJD in inbred strains of mice have provided evidence that agents of BSE and $_{nv}$ CJD are very similar and BSE is therefore the likely source (Bruce *et* al., 1997). Current research is consequently directed at determining the ultimate size of the epidemic and how humans may have become exposed. Research has also stimulated an interest in potential therapeutic strategies and means of diagnosis of the infection in its pre-clinical stage.

The nature of the infectious agent responsible for the TSEs

The nature, and more specifically the molecular structure, of the infectious agent of the TSEs has been the subject of much scientific debate and at times controversy, but at present it remains unknown. An accepted aspect is that one important component of the disease-inducing agent is a post-translational form of the normally produced PrP protein. This normal form is conventionally abbreviated to PrPc, the superscript "C" standing for the normal cellular form. The abnormal form associated with disease is, perhaps unfortunately, referred to under a number of abbreviations: *PrPlc,* PrP's' and PrPRE' - the superscripts referring to scrapie, BSE and resistance to enzymatic, proteinase-K digestion respectively. The last of these characteristics is acknowledged as a valid discriminatory test between the normal and abnormal forms of the PrP protein.

There are two basic hypotheses as to the nature of the infectious agent associated with TSEs. One is that the infectious agent consists of only the modified form of the normal cellular protein. whose function is still not known. This hypothesis was originally outlined by LS. Griffiths and later adoptedby Stanley Prusiner, who in 1982achieved biochemical purification of the scrapie agent. This he termed a "prion", equivalent to a "prion protein", which was considered to be the major component of the infectious agent.

The other main hypothesis is that the infectious agent contains an information molecule, if not a nucleic acid. There are two subhypotheses within this, one being the virus model and the other a virino model. The adherence to these hypotheses is mainly a result of the acknowledged occurrence of strains of the TSE agents, as determined by transmission to specific inbred strains of mice.

The research and debate on the nature of the TSE agent will undoubtedly continue for a few more years. Supporters of the virus and virino hypotheses await the finding of a nucleic acid

or a component that has a similar function. Proponents of the protein-only hypothesis are seeking evidence to explain how strain variation and mutation can be based on a post-translationally modified, normal protein.

Epidemiology

BSE was first recognized in the United Kingdom in 1986 as a result of the routine animal disease surveillance activities. The initial epidemiological studies and histological examination of archived bovine brains indicated that the first cases occurred around 1985. **The detection of BSE was probably aided by the relatively high degree of active communication between animal keepers and veterinarians,** who in turn seek help from the network of Veterinary Investigation Centres, whose staff may seek specialist advice from the Central Veterinary Laboratory. In amore practical sense the disease was identified by its unusual clinical presentation, by the fact that one of the earliest affected herds was very large and multiple cases occurred, and by talk among herdowners about their "unusual" animals, which stimulated the owners to seek veterinary attention (Wilesmith, 1996a). Although **only a small number of cases had been confirmed by histological examination by early 1987** (Wells *et al.,* 1992), the epidemiological study primarily to investigate the potential aetiologies was started in June 1987.

Early studies on the possible aetiology:

The early studies relied on making veterinarians involved in cattle medicine aware of the clinical signs and requesting their voluntary notification. Although the pathological features were reminiscent of scrapie, other potential aetiologies were not excluded. Vehicles of a scrapie-like agent included vaccines, hormones and other biological products, direct or indirect contact with sheep and a variety of free-living animals, imported cattle and semen and feedstuffs containing animal-derived products. The other main potential aetiology was a toxic phenomenon resulting from the use of agricultural chemicals, such as herbicides and pesticides, and pharmaceutical products including organophosphorus preparations, synthetic pyrethroids and anthelmintics. A solely genetic origin was also investigated.

All of the possible vehicles, except feedstuffs, and the other actiologies, were eliminated by December 1987 following the completion of a case study of nearly 200 affected herds and cases of BSE (Wilesmith *et al.,* 1988). With respect to feedstuffs, two possible vehicles of infection were evident: meat and bone meal and tallow. The balance of evidence showed that meat and bone meal was the primary vehicle and therefore responsible for most, if not all, cases.

The suggestion that meat and bone meal was the primary vehicle was based initially on a consideration of the physicochemical properties of the scrapie agent which make it more likely to partition with the protein fraction rather than the lipids of tallow. The geographical variation in incidence and the geographical distribution and handling of tallow compared with meat and bone meal supported the latter. In the early stages of the epidemic, although BSE occurred simultaneously throughout the United Kingdom, the incidence was

markedly greater in the south of England (see Figures 5 and 6). Unlike meat and bone meal which, like proprietary feedstuffs, has a relatively local distribution, tallow is purchased and mixed by a relatively small number of companies which are much more distant and which distribute the product nationally. A similar incidence across the country would therefore have been expected if tallow was the primary vehicle of infection. The reasons for the north-south difference in incidence were identified in a subsequent stage of the epidemiological study and are described later.

The feedborne hypothesis in general was supported by particular features of the epidemic. The incidence of BSE is considerably greater in dairy herds than in beef suckler herds. Commercial feedstuffs containing meat and bone meal are less frequently used to feed beef stickler herds than other animals. In addition, the risk of a herd experiencing a case of BSE increased with increasing herd size. This was consistent with the fact that the larger the herd the more feed is required and the greater the chances of buying an infected batch of feed. In order to investigate the meat and bone meal hypothesis more formally, a case-control study was initiated in early 1988 by recruiting unaffected herds for potential controls, before the hypothesis had become widely known. The final analysis of this study was completed in 1991, allowing for the long incubation period, and the results provided supporting evidence that meat and bone meal was the source (Wilesmith, Ryan and Hueston, 1992).

The initial findings were considered sufficiently strong to make suspicion of the disease statutorily notifiable in June 1988 and ban the feeding of ruminant protein to all ruminants in July 1988 (HMSO, 1988a; 1988b). Subsequently, a standard epidemiological questionnaire has been completed for all suspect cases of B SE reported and the brain of every animal slaughtered has been examined histologically. These measures have resulted in a large and valuable epidemiological database which has facilitated a detailed monitoring of the epidemic.

The Origin of BSE

The working hypothesis for the origin of BSE is sheep scrapie (Wilesmith *et* al., 1988; Kimberlin, 1993; Kimberlin and Wilesmith, 1994; Kimberlin, 1996). This hypothesis arises from the **original considerations with respect to the reason for only the cattle population in the United Kingdom experiencing a major incidence of BSE** (Wilesmith and Wells, 1991). The risk factors originally identified were fourfold: a **large ratio of sheep to cattle population, approximately 4: 1, larger than in any other country; a sheep population endernically infected with scrapie,** although the scrapie status of the sheep population in the United Kingdom may not be unique in this respect (this aspect on its own was unlikely to be important without the presence of the other risk factors); the feeding of ruminant-derived protein to cattle; and conditions of rendering which allowed the effective exposure of the cattle population to a scrapie-like agent.

An examination of these risk factors in countries outside the United Kingdom indicated that they were not present in combination anywhere else, such that a major epidemic of BSE was unlikely. For example, **in France the ratio of cattle to sheep is the reverse of that in the United Kingdom, although the prevalence of sheep scrapie infection may be quite**

similar. Similar risk assessments have been made for the occurrence of BSE in Argentina (Schudel *et* al., 1996) and the United States (Walker *et.* al., 1991; Bleem *et* al., 1994). The risks of BSE occurring in other countries is discussed in a little more detail in Chapter 4.

This international comparison of the above basic risk factors together with the key features of the epidemiology of B SE in the United Kingdom **provide supporting evidence for the sheep scrape origin hypothesis.** However, alternative hypotheses of the origin of BSE have attracted considerable attention, **particularly those related to a possible cattle origin** (see Wilesmith *et* al., 1988; Kimberlin, 1993; Kimberlin and Whilsmith, 1994; Kimberlin, 1996), which are summarized here.

The major requirement for a cattle-origin hypothesis to be consistent with the start and subsequent development of the BSE epidemic is that a geographically widespread reservoir of infection was naturally maintained in the cattle population of the United Kingdom. This infection was essentially non-pathogenic within the commercial life span of cattle although rare sporadic disease could have occurred at an incidence below the inevitable detection threshold of the routine animal disease surveillance system present in the United Kingdom. A further requirement is that changes in rendering conditions that led to sufficient feedborne exposure of cattle to initiate the epidemic converted the avirulent infection into one that produced clinical disease in a large number of animals.

If one considers the hypothesis that BSE has occurred as a sporadic disease at low incidence comparable to that of sporadic CJD in human populations around the world, this **would result in an annual rate of one case per million cattle**. By definition this incidence would be geographically widespread and would result in four cases per year in the United Kingdom. However, the probability of such undetected cases being incorporated into meat and bone meal used in cattle feed would have varied between 30 and 10 percent, depending on the season of the year. It is unlikely therefore that a naturally occurring sporadic incidence of BSE in the cattle population could generate an epidemic because of the improbability of such a low prevalence of infected cattle tissues being recycled via meat and bone meal and being fed to cattle. Indeed the necessary amplification factor for cattle-to-cattle transmission by this means is present in countries such as the United States, and the absence of BSE there is important in this respect. **The hypothesis of sporadic, CJD-like occurrence of BSE in cattle is therefore not consistent with the findings**.

Consideration of the other major contending bovine-origin hypothesis -that of a geographically widespread, high-prevalence avirulent infection of cattle - requires an appreciation of the current, accepted knowledge of the pathogenesis of scrapie and related TSEs. The first aspect of this accumulated research is that the occurrence of infected carriers that do not develop disease has long been suspected to play a role in natural scrapie (Kimberlin, 1993). They are an inevitable consequence of the modal and median age of onset of scrapie, three to five years, which is close to the commercial life span of most breeds of sheep, and naturally subject to the competition from other fatal diseases or those requiring premature culling. Carriers of the scrapie agent are to be expected in sheep because, besides the incubation period, the occurrence of clinical disease depends on two interacting risk factors: the strain of the agent and the genotype of the host. Most of the scrapie strains isolated in mice and hamsters are neuro-invasive and neuropathogenic, but there are major differences in the incubation period depending on the strain of the agent and the genotype of the host. An extreme case is a scrapie model in mice, the 87V strain (Bruce, 1985; Collis and Kimberlin, 1985). In this model, the intraperitoneal injection of low doses into mice can result

in early infection and replication in the lympho-reticular system, but the recipient mice do not develop clinical scrapie in their normal lifespan. **This is because the 87V strain of the scrapie agent cannot migrate from the lympho-reticular system to the central nervous system**. Direct inoculation of the 87V strain into the central nervous system does, however, result in replication. This model is worthy of consideration in the bovine-origin hypothesis of BSE, specifically how such a stable carrier state could be broken.

There are two ways a stable carrier state could be broken. A germ-line mutation in the PrP gene could allow an avirulent strain of the scrapie agent to become neuro-invasive or neuropathogenic. This is unlikely as there is no evidence that the bovine PrP gene exhibits the polymorphisms reported in sheep, mice and humans, and none is associated with the occurrence of BSE. **As indicated in the previous section, there is no significant genetic influence in the susceptibility of cattle to BSE.**

A more likely explanation is a mutation of the avirulent agent to produce a virulent strain. The geographically widespread distribution of the earliest BSE cases requires that such a mutation occurs quite often in cattle.

However, the **cattle-origin hypothesis is not supported by the fact that there have been no major epidemics in other countries**. It would have been expected that the international trade in breeding cattle over decades would have resulted in avirulant endemic infection of cattle in other countries, but this has not occurred. Investigating the origin by means of laboratory-based studies is relatively complex, but long-term studies involving the oral exposure of cattle to sheep scrapie are in progress.

4. Publications of Dr.David BROWN (Lecturer in biochemistry at the Department of Biology and Biochemistry, Bath University and BBSRC Senior Fellow); about the BSE

a/ Discuss a re-evaluation of the TSE enigma and explore the role of environmental factors in prion diseases (2003)

Susan HAYWOOD, BVSc, PhD, MRCVS and David R. BROWN, M.Sc, Ph.D.:
TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES**, VETERINARY TIMES Volume 33, number 2, 27th January 2003**

Dr Susan Haywood is a **Senior Fellow in the Department of Veterinary Pathology, University of Liverpool** with a Wellcome Trust-funded research project into copper pathobiology and proteomics in sheep.

Dr David Brown is a **Lecturer in biochemistry at the Department of Biology and Biochemistry, Bath University and BBSRC Senior Fellow** who has been researching prion diseases for the last ten years. He has a distinguished international reputation in the prion disease field and is invited to lecture on the subject around the

world… (http://www.bath.ac.uk/bio-sci/brown.htm)
(http://www.bath.ac.uk/~bssdrb/Brown.html)

Just over two decades ago the spongiform encephalopathies, as they were then known, were confined to a disparate group that included scrapie in sheep, the rare Creutzfeld-Jakob disease in man (CJD) and the even more exotic Kuru in a supposedly cannibalistic tribe in Papua, New Guinea. All that changed in 1986 when Bovine Spongiform Encephalopathy (BSE) was identified in UK cattle.

Very soon after, the transmissible spongiform encephalopathies (TSE's) or prion diseases came to include transmissible mink encephalopathy (TME), chronic wasting disease in mule deer (CWD), reports of TSE's in zoo animals and felines and latterly variant vCJD in young people. All these are progressively degenerative diseases of the central nervous system that prove ultimately fatal. They are characterised by a long incubation period, failure to elicit an immune response and an aetiology which involves a hitherto unknown class of infectious agents of remarkable stability and persistence. They have in common a pathology that, in addition to neuronal death and spongiform changes, includes the presence of amyloid plaques in the CNS.

The TSE's have raised considerable public concern with respect to the unknown extent of the infection in the food chain, the possible transmissibility to humans and most particularly the relationship of BSE to vCJD. Despite extensive research and an equally wide-ranging BSE Public Inquiry chaired by Lord Phillips (1), there is much that is unanswered or mainly speculative and it is time for a re-evaluation of the collated information, together with more recent investigations which have an important bearing on the pathogenesis on this unique class of diseases.

Scrapie, prion hypothesis and interraction with the host genome

Prior to 1986 most research centred on Scrapie. This disease has been endemic in UK for over 250 years and is found in most countries except Australia and New Zealand. As all Vet students know scrapie is characterised by neuronal vacuolation, reactive astrogliosis and occasional plaque formation. The scrapie agent was deemed infectious in that it could be passaged to other hosts, but was biologically unique in its heat resistant properties, small size and apparent lack of nucleic acid.

As many as 22 strains have been isolated characterised by differing incubation periods in the natural host and lesion profiles produced in inoculated experimental mice. Natural infection is by lateral spread and possibly maternal transmission, otherwise it can be transmitted by intracerebral inoculation to goats, mice and hamsters but not cats or mink. The route of natural infection has been established as via the gastrointestinal tract to the lymphatic tissues (Peyers patches) then to spleen and thymus during which passage the scrapie agent replicates principally within the follicular dendritic cells of the lymphoid tissues. **The subsequent passage to the brain was thought to be via the ganglia of the autonomic system - a process taking many months.**

The cause has been much disputed, but in 1982 Prusiner (2) showed that the scrapie agent was a small proteinaceous infectious particle, lacking nucleic acid,

which he named a prion. He later demonstrated that prion protein PrP^{C} is a component of the normal cell and encoded by the host genome.

The scrapie-associated prion protein PrP^{Sc} has a similar amino acid structure but an altered 3D configuration. This proposed conformational change conferred on it a resistance to protease digestion and the ability to convert normal PrP^{C} to PrP^{Sc}, by a form of chain reaction. The abnormal isoform accumulates in the brain in insoluble aggregates: a characteristic of prion diseases in general. Prusiners hypothesis has become known as the 'protein only' or prion hypothesis, of TSE disease causation.

Genetic factors, however, can affect susceptibility or otherwise to disease, and by 1986 it was understood that susceptibility to scrapie was controlled by a complex interaction between host genes and the particular strain of PrP^{Sc} . The PrP (sinc) gene has been identified in sheep and found to have polymorphisms at codons 136,154 and 171 which are 'disease linked'. In Suffolk sheep it has been found that valine (V), arginine (R) and glutamine (Q) or (VRQ) encoded at these sites are most susceptible to Scrapie, whilst alanine (A), arginine (R), arginine (R) or (ARR) are most resistant. An ARQ associated genotype is also linked with susceptibility in the Suffolk, but not in the Cheviot, breed in which the same pattern confers resistance (3). The underlying rationale of these sometimes conflicting observations is unclear and indeed Phillips warns that "understanding of these polymorphisms is fundamental to efforts to breed scrapie resistant flocks".

BSE not a form of scrapie

Identified in 1986, BSE rapidly spread to affect UK herds although the incidence was very limited within individual herds. The source was assumed, on epidemiological grounds, to be from commercial feed which contained rendered animal protein. Certainly, cases dramatically declined subsequent to the time when the 'ruminant-derived protein ban' came into force in 1988, establishing this theory beyond reasonable doubt.

Initially, the infectious prion was thought to be a modified scrapie prion which had crossed species, but this was later dismissed by Phillips on the ground that BSE differed essentially from scrapie in disease-profile, incubation and transmissibility. The report states with confidence that "the BSE agent is not an unmodified form of scrapie. Rather, it seems to be a novel TSE agent that arose from a prion mutation in cattle, sheep or another species in the 1970's or earlier" (since zoo animals had contracted the disease).

The report goes on to say: "The infectious agent is a post-translationally modified prion protein, a self replicating protein. Other as yet unknown factors may contribute to the development of BSE in infected animals*"*. The source of the supposedly infected feed, however, was never identified and the disease never reproduced experimentally in this way. Also the limited nature of the infection, localised often to just one animal in a herd, was puzzling, the more especially since this could not be explained by host predisposition, as with sheep to scrapie, since no variant polymorphisms have been identified in cattle.

The explanation rests on the unsatisfactory 'packet theory' of infection whereby single high titre doses were unevenly dispersed in the feed; but this has never been confirmed and is at odds with the known high infectivity of PrP^{Sc} affected tissues. Once again, and referred to by Phillips, **other environmental factors may play an aetiological role.**

Link between BSE, and vCJD remains to be confirmed

Human prion disease, including CJD, had been recognised by this time as falling into 3 categories: sporadic (85%), familial (<15%) as a result of a point mutation in PrP gene and iatrogenic (<1% due to medical introduction as a result of vaccines and so on). Epidemiological studies from around the world had moreover failed to identify a causal link between scrapie and CJD. With the emergence of BSE a CJD Surveillance Unit was established with the remit of studying cases of CJD that could have been linked to BSE.

In 1995, two cases of CJD were reported in young people. By March 1996 that had risen to 10 cases. Neuropathological findings revealed the presence of large amyloid plaques in the brains of these unfortunate people, more akin to Kuru than sporadic CJD, and which was now renamed variant CJD . Several studies showed similarities between BSE, vCJD and TSE's in zoo animals and felines. Another study showed that in transgenic mice in which the host PrP gene was replaced by that of human PrP gene (effectively bypassing the species barrier), when challenged with either BSE or vCJD it showed similar patterns of disease distinct from sporadic or iatrogenic CJD (4) .

This is supportive evidence to the effect that BSE and vCJD are caused by a similar strain of prion *but* does not conclude that vCJD is caused by BSE, as Phillips implies.

Circumstantial evidence linking the consumption of beefburgers by young people in support of the transinfection theory, whilst persuasive, has never been proven, in that the putative 'infectious' burgers have never been identified, nor indeed fed, to experimental animals. Groups supposedly more at risk such as farmers, vets, abattoir workers and butchers have not shown an increased risk of vCJD.

It is quite surprising that the one experiment that would confirm a link between BSE and vCJD has not been carried out. If BSE and vCJD are the same strain of disease and take on different characteristics dependent on the host organism, then infecting cows with vCJD should lead to the cows developing BSE.

This would prove BSE and vCJD to be the same disease. However, those who could have carried out the experiments have classed them as "unethical" because of the need to inject human brain into an animal. It is incontrovertible that, the experiments that are the main support for the hypothesis that vCJD and BSE are the same disease also require that vCJD be injected into the brain of the putative source 'host' animal.

Until this is performed, hypotheses of the causal relationship of BSE and other TSE's, including vCJD, remain conjectural and the role of other, possibly environmental, factors must be reconsidered.

The prion 'protein only' hypothesis may require modification

The prion hypothesis suggests that an abnormal isoform of the prion protein PrP^{Sc} alone causes the TSEs. This hypothesis still remains controversial because of the lack of formal proof. In the UK in particular, many scientists still don't accept that this protein is the sole cause of the disease.

Indeed 'manufactured' PrP^{Sc} failed to induce prion disease in mice (5). It is clear, however, that conversion of the normal brain PrP^{C} to the disease specific PrP^{Sc} is necessary for the establishment of the characteristic neuropathology of the TSEs.

PrP is a copper-binding protein with a role in copper metabolism

On the contrary it is almost universally accepted that the normal protein, PrP^{c} is a metal binding protein. PrP^{c} was first suggested to be a copper binding protein in the early 1990's and confirmed by David Brown and colleagues in 1997(6) This has been followed by close to a 100 publications that have reafffirmed that PrP^{c} is a copper binding protein.

Further work by Brown and others showed that PrP^{c} displays SOD-like activity and indeed, it has been proposed that neurodegeneration in prion disease is a direct consequence of a failure of neuronal antioxidant activity. PrP^{c} has a role in cellular copper transport and the sequestration of copper (6). Early work proposed a link between copper metabolism and prion disease in that the copper chelator cuprizone causes neuropathological changes in rodents similar to that seen in experimental scrapie. In this context it is interesting that the abnormal isoform, $PrP^{Sc,}$ is almost devoid of copper and SOD-like activity is severely reduced.

PrP^{Sc} binds manganese in place of copper

A number of researchers have shown that the isoform of the protein associates with other metals. Brown and co-workers have shown that manganese can replace copper in native PrP^{C} to create the isoform PrP^{Sc},. which is protease resistant and lacks antioxidant function, (although there is no evidence that it is infectious).

Most particularly, manganese has been shown to be associated with PrP^{Sc} in the brains of both human CJD patients (7) and mice inoculated with scrapie (8). There is additional evidence that there are changes in metal metabolism in the brain with a loss of brain copper (but an increase in the liver) coincident with the increase of brain manganese in the CJD patients and the scrapie infected mice.

Additionally, manganese elevations in rodent scrapie are both presymptomatic and systemic. The cause of this remains unclear, but it is currently being investigated as to whether elevated blood manganese can be used as a diagnostic test for TSEs. All in all, there is a solid and expanding amount of literature showing that metal imbalance and TSEs are linked.

TSE's may be an environmental/industrial disease

Coming at these diseases from another direction, **Mark Purdey, a farmer** from Somerset, has published evidence that hotspots of TSEs exist in regions of the world **where there is environmental imbalance between copper and manganese.**

Farms in Iceland prone to scrapie have soils with dramatically increased manganese levels. A similar situation exists in Colorado where deer develop chronic wasting disease (9). These findings led both Mark **Purdey and David Brown** to **hypothesise that sporadic TSEs might be a result of animals becoming exposed to conditions where manganese in their diets is elevated and copper is deficient.**

Manganese and copper are ubiquitous metals and it is hard to imagine how the changes in these metals might initiate such diseases. Since the mid 20th Century, however, industrialisation has been fairly intensive, especially in the ferromanganese industry and considerable amounts of manganese are present in pollution. Indeed lead fuel replacements also use manganese. In Slovakia, there are high levels of both inherited and sporadic forms of CJD around areas of intense ferromanganese industry. Therefore there is the potential for new disease to arise as a result of intense industrialisation.

One issue with BSE and vCJD remains unanswered: why such a high incidence in the UK? One possibility is that the UK is just the first, and others will follow. BSE that was once thought to be specific to UK is now Europe wide.

In the UK, the majority of BSE developed as a result of the feeding of offal from BSE-infected animals back to other animals. This unfortunately has masked the possibility of tracing the origins of BSE back to any source.

Similarly, the mobility of humans around the UK also makes it difficult to trace vCJD back to any factor, especially not one that might be 'environmental'.

Although the BSE Inquiry clearly stated that alternative possibilities for the origin of BSE should be investigated and given support, there has been little support forthcoming, despite the fact that most of the evidence linking TSEs and manganese has appeared since that time.

This is in part as a result of the subsequent Horn report "*Review of the origin of BSE"* (10). This report failed to note that comparing maps of BSE incidence to a map of manganese hotspots across the UK when the epidemic was well established was inappropriate, since BSE was clearly spread at this stage by recycling infected offal.

A more detailed analysis, looking at the location of the very first cases of where BSE were reported (as viewed on the DEFRA web page,) and the map provided in the Horn report**, indicates that the original BSE farms lie directly in a manganese hot spot!**

Others, however, have not allowed themselves to be side tracked in this way but concentrated on the scientific evidence. In particular, authorities in the Environmental Protection Agency in Colorado have begun investigating the link between manganese, copper and chronic wasting disease incidence in deer. This disease was originally thought to be a copper deficiency disease before the prion hypothesis came to be recognised and CWD was recognised as a TSE.

Although manganese might not be 'the cause' it is clear from the biochemical studies that have been carried out that metals do play a role in the pathogenesis of TSEs. Therefore, even if manganese is just a risk factor, it is important that this factor be kept in the equation, because it might just be the key that unlocks the truth about these diseases.

Finally, the most tantalising question remains: how does the PrP^{Sc} isoform propagate and, even more so, become infectious? It has been shown that the manganese-bound isoform PrP^{Sc} has a configuration that makes it protease resistant and that this particular conformation tends to form spontaneous amyloid aggregates.

It is suggested that when this self-propagation reaches an optimum size, it recruits normal cellular PrP^{C} expression to convert to PrP^{Sc} which then replicates uncontrollably: a sort of hijacking of the synthetic machinery which then operates in a runaway fashion, free of normal checks and balances.

It is interesting that metalloprotein chemistry of this nature (but involving metals other than manganese) is now being implicated for the analogous plaque formation in Alzheimer's and certain other neurodegenerative conditions. If so, then the prion diseases are not a biological freak of nature but a subclass of a category of diseases relating to dysregulation of metalloprotein chemistry associated with metal imbalance.

References

(1) *The BSE Inquiry Report*: (2000).
(2) Prusiner S.(1982) Novel Infectious agents Cause Scrapie. *Science* 216,136-144
(3) Hunter N, Foster J, Goldman W, Stern M, Hope J & Bostock C.(1996) Natural Scrapie in a Closed flock of Cheviot Sheep occurs only in specific PrP genotypes. *Archives of Virology* **141:**809-824
(4) Hill AF, Debruslais M, Joiner S, Sidle KC, Gowand I, et al (1997) The same prion strain causes vCJD and BSE. *Nature*, **389**:448-50
(5) Hill AF, Antoniou M, Collinge(1999) J.Protease resistant prion protein produced *in vitro* lacks detectable infectivity. *J Gen Virol*. **80**:11-14.
(6) Brown D R (2001) Copper and prion disease. *Brain Research Bulletin*. **55** (2): 165-173.
(7) Boon-Seng Wong, Shu G Chen, Colucci M, Xie Z, Pan T, Liu T Li R Gambetti P, Sy M-S & Brown D R. (2001) Aberrant metal binding by prion protein in human prion disease. *Journal of Neurochemistry*, **7:**1,400-1,408.
(8) Thackrey A M, Knight R, Haswell S J, Bujdoso R & Brown D R (2002) Metal imbalance and compromised antioxidant function are early changes in prion disease. *Biochem J.* **362**: 253-258.
(9) Purdey M. (2000) Ecosystems supporting clusters of sporadic TSE's demonstrate excesses of the radical generating divalent cation manganese and deficiencies of antioxidant co-factors; Cu, Se, Fe, Zn. Does a foreign cation substitution at ar PrP's Cu domain initiate TSE? *Med. Hypoth*. **54**:278-306.
(10) Horn, G. (2001) *Review of the Origin of BSE.*

b/ BSE did not cause variant CJD; an alternative cause related to post-

industrial environmental contamination (BROWN,2001)(http://www.ela-europe.org/ecritcms/getfile.php/BSE%20did%20not%20cause%20variant%20CJD:.pdf?id=30,9b769)

Until the suggestion that the disease BSE passes to humans in the form of vCJD there was no evidence that animal disease can cause human prion disease. Indeed, the existence of a species barrier (COLLINGE et al., 1995) 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 occuring.

BSE is claimed to have occured as a result of a subtype of scrapie infecting cattle through ingestion of contaminated offal (BAKER and RIDLEY, 1996). 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 vCJD 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 suggest 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.

Scientific proof that BSE causes vCJD rests upon the fact that BSE was first diagnosed 10 years before vCJD was diagnosed. However, there is no proof of how long subclinical BSE and vCJD existed in the UK before this time. The report of an individual diagnosed with vCJD 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 vCJD only coincidentally supports the notion that BSE caused vCJD, 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*** *(BRUCE et al.,1997; COLLINGE et al., 1996; SCOTT et al., 1999; HILL et al., 1997). 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, vCJD cases produce a single profile in mice expressing the human prion protein. When the same mice are infected with BSE, the same profile as vCJD emerges.*

PrPSc has three forms depending on glycosilation. PrPSc is identified as such by digestion of brain extracts with proteinase K. The elctrophoretic 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, vCJD has a specific pattrn of glycoforms that is unlike those of sporadic CJD. However, the glycoform patern produced by vCJD and bSE injected into mice is similar.

No matter how convincing this might sound, these results do not demonstrate that BSE caused vCJD. If the results as described are accepted they simply show that BSE and vCJD are the same „strain" of prion disease. However, it is not really clear what a strain is or how it is caused. Furthemore, 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 vCJD. 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 „confortable" to assume that similarities in the disease profiles of mice infected with either BSE or vCJD imply that these diseases are „similar", the fact is, a mouse is not cow and it is not a human. Condensing a disease that takes 20 years in human or 6 years in a cow to a few months in a mouse will certainly abolish any subtle differences that would otherwise distinquish them. One such difference is the occurence 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.*

Perhaps vCJD 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 vCJD 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. Threfore 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.

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. Sueprisingly, this phenomenon cannot be associated with particular strains of sheep and when the sheep on a particular scrapie- prone ferm are slaughtered and the the vegetation on the farm replaced , new stock develop scrapie with the same incidence. Additionally , CWD the disease of deer in the USA, also shows a regional distribution.

A link between metals and prion disease is more supportable. Recent evidence suggests that PrPc can bind either cooper or manganese (BROWN et al., 1997; BROWN et al., 2000). However, on binding manganese the protein is more likely to change its secondary structure to one resembling PrPSc (BROWN et al., 2000). Incorporation of manganese in place of copper can cause conversion of PrPc into PrPSc. Cells cultured in media with high levels of manganese disolved in the medium show increased expression of proteinase K resistant PrP (BROWN et al., 2000).

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.

I propose that ***the new prion diseases are postindustrial phenomena which will spread through the world in relation to the time*** *and extent of industrialization in the countries of the world….*

References

BAKER,H.F.- RIDLEY,R.M.; What went wrong in BSE? From prion disease to public disaster. Brain Res.Bull., 40, 1996; 237-244

BROWN,D.R.- QUIN,K.- HERMS,J.W. et al.: The cellular prion protein binds copper in vivo. Nature, 390, 1997; 684-687

BROWN,D.R.- HAFIZ,F.- GLASSSMITH,L.L. et al.: Consequences of manganese replacement of copper for prion protein function and proteinase resistance. EMBOJ, 19, 2000; 1180-1186

BROWN,D.R.: BSE did not cause variant CJD; an alternative cause related to post-industrial environmental contamination. Medical Hypotheses, 57 (5), 2001; 555-560

BRUCE,M.- WILL,R.G.- IRONSIDE,J.W. et al.: Transmissions to mice indicate that „new variant“ CJD is caused by the BSE agent. Nature, 389, 1997; 498-501

COLLINGE,J.-PALMER,M.S.- SIDLE,K.C. et al.: Unaltered susceptibility to BSE in

transgenic mice expressing human prion protein. Nature, 378, 1995; 779-837
COLLINGE,J.- SIDLE,K.C.L.- MEADS,J. et al.: Molecular analysis of prion strain variation and the aetiology of new variant CJD. Nature, 383, 1996; 685-690
HILL,A.F.- DESBRUSLAIS,M.- JOINER,S. et al.: The same prion strain causes vCJD and BSE. Nature, 389,1997; 448-450
SCOTT,M.R.- WILL,R.- IRONSIDE,J. et al.: Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc.Natl.Acad.Sci.USA, 96, 1999; 15137- 15142

Publications by David Brown related to the prion protein or prion disease

1.. Brown, D. R., Herms, J. and Kretzschmar, H. A. (1994) Mouse cortical cells lacking cellular PrP survive in culture with a neurotoxic PrP fragment. Neuroreport 5: 2057-2060.

2. Brown, D. R., Schmidt, B. and Kretzschmar, H. A (1996) Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature 380: 345-347.

3. Brown, D. R., Schmidt, B. and Kretzschmar, H. A (1996) A neurotoxic prion protein fragment enhances proliferation of microglia but not astrocytes in culture. Glia 18: 59-67.

4. Kretzschmar, H. A., Giese, A., Brown, D. R., Herms, J. W., Schmidt, B. and Groschup, M. H. (1996) Cell Death in Prion Disease in Transmissible Subacute Spongiform Encephalopathies: Prion Diseases. Ed., Court, L. and Dodet, B., Elsevier, Paris, pp: 97-106.

5. Kretzschmar, H. A., Giese, A., Brown, D. R. Herms, J. W., Keller, B. Schmidt, B. Groschup, M. (1997) Cell Death in Prion Disease. J. Neural Transm. [Suppl] 50: 191-210

6. Brown, D. R. and Kretzschmar, H. A. (1997) Microglia and prion disease: a review. Histol. Histopathol. 12: 883-892.

7. Brown, D. R., Herms, J. W., Schmidt, B. and Kretzschmar, H. A (1997) Different requirements for the neurotoxicity of fragments of PrP and ß-amyloid.
Euro. J. Neurosci. 9: 1162-1169.

8. Herms, J. W., Madlung, A., Brown, D. R. and Kretzschmar, H. A. (1997) Increase of intracellular free Ca2+ in microglia activated by prion protein fragment. Glia 21: 253-257.

9. Brown, D. R., Schulz-Schaeffer, W. J., Schmidt, B. and Kretzschmar, H. A (1997) Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. Exp. Neurol. 146: 104-112.

10. Brown, D. R., Qin, K., Herms, J. W., Madlung, Manson, J., Strome, R., Fraser, P. E. Kruck, T., A., von Bohlen, A., Schulz-Schaeffer, W., Giese, A., Westaway, D. and Kretzschmar, H. (1997) The cellular prion protein binds copper in vivo. Nature 390:684-687.

11. Brown, D. R., Schmidt, B. and Kretzschmar, H. A. (1997) Expression of prion protein in PC12 is enhanced by exposure to oxidative stress. Int. J. Dev. Neurosci. 15: 961-972.

12. Kretzschmar, H. A., Windl, O., Brown, D. R., Giese, A., Schulz-Schaeffer, W. and Herms, J. (1998) Molecular pathology of transmissible spongiform encephalopathies. Neurosci. News 1: 17-25.

13. Brown, D. R., Schmidt, B., Groschup, M. H. and Kretzschmar, H. A. (1998) Prion protein expression in muscle cells and toxicity of a prion protein fragment. Eur. J. Cell Biol. 75: 29-37.

14. Brown, D. R., Schmidt, B. and Kretzschmar, H. A. (1998) A prion protein fragment interacts with PrP-deficient cells. J. Neurosci. Res. 52: 260-267.

15. Brown, D. R., Schmidt, B. and Kretzschmar, H. A. (1998) A prion protein fragment primes type 1 astrocytes to proliferation signals from microglia. Neurobiol. Disease 4: 410-422.

16. Brown, D. R., Schmidt, B. and Kretzschmar, H. A. (1998) Effects of copper on survival of prion protein knockout neurones and glia. J. Neurochem. 70, 1686-1693.

17. Brown, D. R., Besinger, A., Herms, J. W. and Kretzschmar, H. A. (1998) Microglial expression of the prion protein. Neuroreport 9, 1425-1429.

18. Giese, A., Brown, D. R., Groschup, M. H., Feldmann, C., Haist, I. and Kretzschmar, H. A. (1998) Role of microglia in neuronal cell death in prion disease. Brain Pathol. 8, 449-457.

19. Brown, D. R., Pitschke, M., Riesner, D. and Kretzschmar, H. A. (1998) Cellular effects of a neurotoxic prion protein peptide are related to its b-sheet content. Neurosci. Res. Comm. 23: 119-128.

20. Brown, D. R. (1998) Toxicity of a b-amyloid peptide fragment in neurones and glia with reduced APP expression. Alzheimer's Rep. 1, 223-231.

21. Brown, D. R. and Besinger, A. (1998) Prion protein expression and superoxide dismutase activity. Biochem. J. 334, 423-429.

22. Brown, D. R. (1998) Prion protein-overexpressing cells show altered response to a neurotoxic prion protein peptide J. Neurosci. Res. 54, 331-340.

23. Brown, D. R. and Mohn, C. M. (1999) Astrocytic glutamate uptake and prion protein expression. Glia 25, 282-292.

24. Wong, B.-S., Wang, H., Brown, D. R. and Jones, I. M. (1999) Selective oxidation of methionine residues in prion proteins. Biochem. Biophys. Res. Comm. 279, 352-355.

25. Brown, D. R. (1999) Prion protein peptide neurotoxicity can be mediated by astrocytes. J. Neurochem. 73, 1105-1113.

26. McHattie, S. J., Brown, D. R. and Bird, M. M. (1999) Cellular uptake of the prion protein fragment PrP106-126 in vitro. J. Neurocytol. 28, 145-155.

27. Bürkle, A., Kretzschmar, H. A. and Brown, D. R. (1999) Poly(ADP-ribose) immunostaining to detect apoptosis induced by a neurotoxic fragment of prion protein. Histochem. J. 31, 711-716.

28. Brown, D. R. (1999) Prion protein expression aids cellular uptake and veratridine-induced release of copper. J. Neurosci. Res. 58, 717-725.

29. Brown, D.R., Wong, B.S., Hafiz, F., Clive, C., Haswell, S. and Jones, I.M. (1999) Normal prion protein has an activity like that of superoxide dismutase. Biochem. J. 344, 1-5.

30. Brown, D. R. (1999) Comment on: Neurotoxicity of prion protein peptide 106-126 not confirmed. FEBS Lett. 460, 559-560.

31. Brown, D. R. (2000) Prion protein peptides: Optimal toxicity and peptide blockade of toxicity. Mol. Cell Neurosci. 15, 66-78.

32. Brown, D. R. (2000) Altered toxicity of the prion protein peptide PrP106-126 carrying the A117V mutation. Biochem. J. 346, 784-791.

33. Wong, B.-S., Clive, C., Haswell, S. J., Jones, I. M. and Brown, D. R. (2000) Copper has differential effect on prion protein with polymorphism of position 129. Biochem Biophys. Res. Comm. 269, 726-731.

34. Brown, D. R., Hafiz, F., Glasssmith, L. L., Boon-Seng Wong, B.-S., Jones, I. M., C Clive, C., and Haswell, S. J. (2000) Consequences of manganese replacement of copper for prion protein function and proteinase resistance. EMBO J. 19, 1180-1186.

35. Brown, D. R. , Iordanova, I. M., Wong, B.-S., Vénien-Bryan, C., Hafiz, F., Glasssmith, L. L., Sy, M.-S. , Gambetti, P., Jones, I. M., Clive, C. and Haswell, S. J. (2000) Functional and structural differences between the prion protein from two alleles prnpa and prnpb of mouse. Eur. J. Biochem. 267, 2452-2459.

36. Hafiz, F. and Brown, D. R. (2000) A model for the mechanism of astrogliosis in prion disease. Mol. Cell Neurosci. 16, 221-232.

37. Post, K., Brown, D. R., Groschup, M., Kretzschmar H. and Riesner, D. (2000) Neurotoxicity but not infectivity of prion proteins can be induced reversibly in vitro. Arch. Virol. Suppl.16, S265-S273.

38. Wong, B.-S., Pan, T., Liu, T., Li, R., Jones, I. M., Gambetti, P., Petersen, R. B., Brown, D. R. and Sy, M.-S. (2000) Prion disease: a loss of anti-oxidant function? Biochem. Biophys. Res. Comm. 275, 249?252.

39. Wong, B.-S., Vénien-Bryan, C., Williamson, R. A., Burton , D. R., Gambetti, P., Sy, M.-S., Brown, D. R., and Jones, I. M. (2000) Copper refolding of Prion Protein. Biochem. Biophys. Res. Comm. 276, 1217-1224.

40. Brown, D. R. (2000) PrPSc-like prion protein peptide inhibits the function of cellular prion protein. Biochem. J. 352, 511-518.

41. Brown, D. R. (2001) Prion protein peptide: agents of death for neurons. in Molecular Pathology of Prion Diseases. Ed. Baker, H. Humana Press Totowa, New Jersey, USA pp.51-70.

42. Brown, D. R. and Jones, I. M. (2001) A function for the prion protein? in Molecular Pathology

of Prion Diseases. Ed. Baker, H. Humana Press Totowa, New Jersey, USA pp:31-50..

43. Brown, D. R., Clive, C. and Haswell, S. J. (2001) Anti-oxidant activity related to copper binding of native prion protein. J. Neurochem. 76, 69-76.

44. Brown, D. R. (2001) Microglia in Prion Disease. Microscop. Res. Tech. 54, 71-80.

45. Wong, B.-S. Liu, T., Ruliang Li, R., Pan, T., Petersen, R. B., Smith, M. S. Gambetti, P., Perry, G., Manson, J.,. Brown, D. R. and Sy, M.-S. (2001) Increased levels of oxidative stress markers detected in the brains of mice devoid of prion protein. J. Neurochem. 76, 565-572.

46. Brown, D. R. (2001) Prion and prejudice: normal protein at the synapse. Trends Neurosci. 24, 85-90.

47. Brown, D. R. (2001) Copper and prion disease. Brain Res. Bull. 55, 165-173.

48. Wong, B.-S., Liu, T., Paisley, D., Li, R., Pan, T. Chen, S. G., Perry, G., Petersen, R. B., Smith, M. A., Melton, D. W., Gambetti, P., Brown, D. R. and Sy, M.-S. (2001) Induction of HO-1 and NOS in doppel-expressing mice devoid of PrP: Implications for Doppel function. Mol. Cell Neurosci. 17, 768-775.

49. Brown, D. R. (2001) BSE: A post-industrial disease?*Chem. Indust.* **3**, 73-76.

50. Brown D. R. (2001) BSE did not cause variant CJD: An alternative cause related to post-Industrial environmental contamination. *Med. Hypoth.* **57**, 555-560.

51. Brown, D. R. (2001) An alternative cause for BSE and variant CJD related to post-industrial environmental contamination. *World Leather* **14**, 33-38.

52. Wong, B.-S., Chen, S. G., Colucci, M., Xie, Z., Pan, T., Liu, T., Li, R., Gambetti, P., Sy, M.-S. and **Brown, D. R.** (2001) Aberrant metal binding by prion protein in human prion disease. *J. Neurochem.* **78**, 1400-1408.

53. Wong, B.-S., **Brown, D. R.** and Sy, M.-S. (2001) A yin-yang role for metals in prion disease. *Panminerva Med.* **43,** 283-287.

54. Wong, B.-S., **Brown, D. R.**, Pan, T., Whiteman, M., Liu, T., Bu, X., Li, R., Gambetti, P., Olesik, J., Rubinstein, R. and Sy, M.-S. (2001) Oxidative impairment in scrapie-infected mice is associated with brain metal perturbations and altered ani-oxidantion activities. *J. Neurochem.* **79**, 689-698.

55. Daniels, M., Cereghetti, G. M. and **Brown, D. R.** (2001) Toxicity of novel C-terminal prion protein fragments and peptides harbouring disease-related C-terminal mutations.*Eur. J. Biochem*. **268**, 6155-6164.

56. Daniels, M. and **Brown, D. R.** (2002) Purification and preparation of prion protein: The synaptic superoxide dismutase. *Meth. Enzymol*. **349**, 258-267.

57. Brown, D. R. (2002) Prion protein: A synaptic cuproprotein *in* Handbook of Copper

Pharmacology and Toxiciology Ed. E. Massaro, Humana Press, Totowa, New Jersey, USA. pp115-129.

58. Brown, D. R., Nicholas, R. St. J., and Canevari, L. (2002) Lack of prion protein expression results in a neuronal phenotype sensitive to stress. *J. Neurosci. Res.* **67**, 211-224.

59. Wong, B.-S., Sy, M.-S. and **Brown, D. R.** (2002) Prion-like doppel protein expression correlates with heme oxygenase and nitric oxide synthase induction. *Heme Oxygenase in Biology and Medicine.* Ed. N.G Abraham, Plenum Press, New York, USA pp. 423-430.

60. Thackray, A. M., Knight, R., Haswell, S. J., Bujdoso, R. and **Brown, D. R.** (2002) Metal imbalance and compromised antioxidant function are early changes in prion disease. *Biochem. J.* **362**, 253-258.

61. Brown, D. R. (2002) Molecular advances in understanding inherited prion diseases. *Mol. Neurobiol.* **25**, 287-302.

62. Brown, D. R. (2002) Don't lose sleep over prions: role of prion protein in sleep regulation. *Neuroreport* **13**, A1.

63. Brown, D. R. (2002) Mayhem of the multiple mechanisms: Modelling neurodegeneration in prion disease. *J. Neurochem.* **82**, 209-215**.**

64. Sigurdsson, E. M., **Brown, D. R**., Daniels, M., Kascsak, R. J., Kascsak, R., Carp, R., Meeker, H. C., Frangione, B. and Wisniewski, T. (2002) Immunization delays the onset of prion disease in mice. *Am. J. Pathol.* **161**, 13-17.

65. Brown, D. R. and Sassoon, J. (2002) Copper dependent functions for the prion protein. *Molec. Biotech.* **22**, 165-178.

66. Ellis, V., Daniels, M., Misra, R. and **Brown, D. R.** (2002) Plasminogen activation is stimulated by prion protein and regulated in a copper-dependent manner. *Biochemistry* **41**, 6891-6896.

67. Wisniewski, T., **Brown D. R.** and Sigurdsson, E. M. (2002) Therapeutics in Alzheeimer's and prion disease. *Biochem. Soc. Trans.* **30**, 574-578.

68. Brown, D. R. (2002) Copper and prion disease. *Biochem. Soc. Trans.* **30**, 742-745.

69. Turnbull, S., Tabner, B. J., **Brown, D. R.** and Allsop, D. (2003) Copper-dependent generation of hydrogen peroxide from the toxic prion protein fragment PrP106-126. *Neurosci. Lett.* **336**, 159-162.

70. Thackray, A. M., Madec, J. Y., Wong, E., Morgan-Warren, R., **Brown, D. R.**, Baron, T. and Bujdoso, R. (2003) Detection of BSE, ovine scrapie PrP Sc and normal PrP c by monoclonal antibodies raised to copper-refolded prion protein. *Biochem J.* **370**, 81-90.

71. Haywood, S. and **Brown, D. R.** (2003) Transmissible spongiform encephalopathies: a revaluation and possible role of environmental factors in prion diseases. *Vet. Times*, **33:2**,

8-10.

72. Sassoon, J. and **Brown, D. R.** (2003) Copper and prion disease. *Metal Ions and Neurodegeneration* Ed. Zatta, P., World Scientific, New Jersey. Pp. 279-306.

73. Cui, T., Holme, A., Sassoon, J. and **Brown, D.R**. (2003) Analysis of doppel protein toxicity. *Mol. Cell Neurosci.* **23**, 144-145.

74. Wong, B. S., Li, R., Sassoon, J., Kang, S.-C., Liu, T., Pan, T. Wisniewski, T., **Brown, D. R.** and Man-Sun Sy. M.-S. (2003) Mapping the antigenicity of copper-treated cellular prion protein with the scrapie isoform. *Cell Molec. Life Sci.* **60**, 1224-1234.

75. Turnbull, S., Tabner, B. J., **Brown, D. R.** and Allsop, D. (2003) Generation of hydrogen peroxide from mutant forms of the prion protein fragment PrP121-231 *Biochemistry* **42**, 7675-7681.

76. Liberski, P. P., Sikorska, B., Bratosiewicz-Wlsik, J., Walic, A., Brown, P., and **Brown, D. R.** (2003) Exuberant cellular reaction of the optic nerves in experimental Creutzfeldt-Jakob disease. *Acta Neurobiol. Exp.* **63,** 309-318.

77. Brown, D. R. and Sinclair, K. (2003) Deer slaughter outrage. *Vet. Times* **33:14,** 18.

78. Holme, A., Daniels, M., Sassoon, J. and **Brown, D. R.** (2003) A novel method of generating neuronal cell lines from gene-knockout mice to study prion protein membrane orientation. *Eur. J. Neurosci*. **18**, 571-579.

79. Turnbull, S., Tabner, B. J., **Brown, D. R.** and Allsop, D. (2003) Quinacrine acts as an antioxidant and reduces the toxicity of the prion peptide PrP106-126. *Neuroreport* **14**, 1743-1745.

80. Cui, T., Daniels, M., Wong, B. S., Li, R., Sy, M.-S., Sassoon, J. and **Brown, D. R.** (2003) Mapping the functional domain of the prion protein. *Eur. J. Biochem.* **270**, 3368-3376.

81. Brown, D. R. (2003) Conformational exposure: a new handle on prions. *Lancet* **362**, 929-930.

82. Brown, D. R. (2003) Prion protein expression modulates neuronal copper content. *J. Neurochem* . **87**, 377-385.

83. Sigurdsson, E. M., **Brown, D. R.,** Alim, M. A., H. Scholtzova, H., Carp, R. H.C. Meeker, H. C., Prelli, F., Frangione, B., Wisniewski, T. (2003) Copper chelation delays the onset of prion disease. *J. Biol. Chem.* **278**, 46199-46202.

84. Brown, D. R. and Sassoon, J. (2004) Role of glia in prion disease. *Advance. Mol. Cell Biol.* **31,** 1085-1104.

85. Brown, D. R., Guantieri, V., Grasso, G., Impellizzeri, G., Pappalardo, G. and Rizzarelli, E. (2004) Copper(II) complexes of peptide fragments of the prion protein. Conformation changes induced by copper(II) and the binding motif in C-terminal protein region. *J. Inorgan. Biochem.* **98**, 133-143.

86. Thompsett, A. R. and **Brown, D. R**. (2004) A functional role for a copper binding prion protein.

Prions and Prion Diseases: Current Perspectives. Ed. Telling, G. pp.1-40. Horizon Bioscience, Wymondham, UK.

87. Sassoon, J. and **Brown, D. R.** (2004) Neuronal death in prion disease. (In Press).

88. Sassoon, J., Banks, F. and **Brown, D. R.** (2004) Neurotoxicity and prion disease. In *Excitotoxicity in Neurological Disease, New Therapeutic Challenge* Ed. Ferrarese, C. and Beal, M. F. pp. 265-283 Kluwer, Hingham USA .

89. Sassoon, J., Sadowski, M., Wisniewski, T. and **Brown, D. R.** (2004). Therapeutics and prion disease: Can immunisation or drugs be effective? Mini-Rev. Med. Chem. (In Press).

90. Sassoon, J., Daniels, M. and **Brown, D. R.** (2004) Astrocytic regulation of NMDA receptor subunit composition modulates the toxicity of prion peptide PrP106-126. *Mol. Cell Neurosci*. **25**, 181-191.

91. Brown, D. R. (2004) Role of the prion protein in copper turnover in astrocytes. *Neurobiol. Dis.* **15**, 534-543.

92. Kang, S-C., **Brown, D. R.** Whiteman, M., Li, R., Pan, T., Perry, G., Thomas Wisniewski, T., Sy, M.-S., and Wong, B.-S. (2004) Prion protein is ubiquitinated after developing protease resistance in the brains of scrapie-infected mice. *J. Pathol*. **203**, 603-608.

93. Brown, D.R. (2004) Metallic Prions. *Biochem. Soc. Symp.* **71**, 193-202.

94. Calissano, M., Ensor, E., Irshad, S., **Brown, D. R.** and Latchman, D. S. (2004) Transcriptional regulation of prion protein like doppel by the Brn-3a and Brn-3b transciptional factors. *Neuroreport* **15**, 483-486.

95. Brown, D. R. and Kozlowski, H. (2004) Biological inorganic and bioinorganic chemistry of neurodegeneration based on prion and Alzheimer's diseases. *Dalton Trans.* In Press.

96. Jones, C. E., Abdelraheim, S. R., **Brown, D. R.** and Viles, J. H. (2004) Preferential copper^{2+} coordination by His96 and His111 induces b-sheet formation in the unstructured amyloidogenic region of the prion protein. *J. Biol. Chem.* In Press.

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5. Other relationships about „MBM infectiosity"

(the opposite to the „WILLESMITH theory" findings in the UK)

The epidemiology of BSE in **Northern Ireland from 1988, when it was first confirmed, to the end of 1995** is described (DENNY and HUESTON, 1997). All cases of BSE were subjected to a detailed epidemiological investigation, complemented by data from the national animal health records on every bovine animal. Data are presented on 1680 cases. Many of the epidemiological features of the disease were similar to those reported in Great Britain, but the incidence in Northern Ireland was approximately one-tenth that in Great Britain. **The epidemic increased to a peak of 56**

cases per month in January 1994, and decreased to nine cases in December 1995. Statutory intervention banning the use of meat and bone meal in ruminant feed in January 1989 has produced a marked and continuing reduction in the incidence. The majority of the cases were in Northern Ireland Cattle, but 83 cases were imported from Great britain and five from the Republic Ireland. Many of the key epidemiological features have remained constant throughout the epidemic; the greater incidence of BSE in dairy herds than in beef suckler herds, the low within-herd incidence, the variation in incidence with herd size, the breed distribution, the distribution of the reported clinical signs and the proportion of purchased cases. **Although the source of the BSE epidemic in Northern Ireland has not been established conclusively**, the evidence suggests that the importation of MBM and protein concentrates from Great Britain may have been responsible.

Fourteen cases of BSE were diagnosed on the basis of clinical examination in a closed herd of British Friesian cows during a 9-month period from October 1987 until June 1988 (WINTER et al., 1989). The diagnosis was confirmed on histopathological examination of brain tissue from five of the six samples submitted. The main presenting clinical signs were of altered behaviour; apprehension, anxiety and hyperaesthesia. One cow was euthanized after a short period of recumbency; the remaining 13 cows were slaughtered on humanne or economic grounds. **No protein of animal origin had been fed to either heifers or cows in this herd during the past 5 years and there had been no direct contact with sheep…**

Evidence that changes in feeding style alter the membrane fatty acid composition of ruminant tissue is presented here by comparing zoo giraffe with the same species from their natural habitat (CRAWFORD et al., 1991). The membrane changes seen are similar to those used experimentally to make animals susceptible to basic brain protein and encephalomalacia. Similar membrane responses have been in cattle. Use of animal protein and **increased nitrogen in cattle feeds would lead to a relative deficiency of essential fatty acids in the cell membranes and hence reduced membrane stability**. By analogy with crazy chick disease (nutritional encephalomalacia) and experimental encephalomyelitis in rats, the possibility that the changes in animals feeds would have depleted cattle tissue membranes and made them susceptible to BSE is discussed. The assumption being made is that the principle of a requirement of essential fatty acids for neural integrity and immune systém function would apply to cattle as well as to other species.

References

CRAWFORD,M.A.- BUDOWSKI,P.- DRURY,P.- GHEBREMESKEL,K.- HARBIGE,L.- LEIGHFIELD,M.- PHYLACTOS,A.- WILLIAMS,G.: The nutritional contribution to bovine spongiform encephalopathy. Nutr.Health, 7(2), 1991; 61-68

DENNY,G.O.-HUESTON,W.D.: Epidemiology of BSE in Northern Ireland 1988 to 1995. Vet.Rec., 1040(2), 1997; 302-306

WINTER,M.H.- ALDRIDGE,B.M.- SCOTT,P.R.- CLARKE,M.: Occurrence of 14 cases of BSE in a closed dairy herd. Br.Vet.J., 145(2), 1989; 191-194

Selenium Deficiency as the cause of BSE

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The Hypothesis

It is postulated that BSE is a disease of selenium deficient cattle which have been fed meat and bone meal containing fragments of bacterial membrane of a composition that releases moieties which can be transported into the brain by the Pr protein.

Selenium deficiency

Selenium is not an essential element for plants, and the amount they contain depends upon its availability in the soil. The element is present as selenate in alkaline and selenite in acid soil, but is probably precipitated as selenide in anaerobic or over-exploited soil (1). It is reasonable to assume that crops grown in the UK at the present time contain less than in the past. Heavy metals, including molybdenum complex with selenium.

In mammals glutathione peroxidase and deiodinase I are seleno-proteins, and deiodinase II is inactivated during selenium deficiency. The relationship between deiodinase I and II is complex, but in rats and humans the levels of triiodothyronine (T3) in blood is stabilised during selenium deficiency by selenium being withdrawn from peripheral tissues to the thyroid and pituitary glands where deiodinase I activity is increased. Neither of the deiodinase enzymes is ever present within the thyroid gland of ruminants, and this is thought to render them more susceptible to selenium deficiency (2).

There are indications that humans who have experienced low intakes of selenium from birth are adapted to low intake, and personal experience with cattle supports this conclusion. It is proposed, therefore, that it is not so much a low level of selenium that inhibits deiodinase II, but the magnitude of any decrease in its availability. This means that when calves that have been reared on a high selenium diet are transferred as adults to pastures which are low in selenium, they are exceptionally susceptible to the consequences of selenium deficiency.

It is suggested that it is the movement of cattle from high to low selenium diets which makes them susceptible to BSE. This also explains the unusual distribution of the disease in which herds seldom contain more than one or two cases of BSE at any one time, and in which on some farms the only cases ever seen are in cows which have been purchased to make up numbers.

Hypomagnesaemia in cattle

Hypomagnesaemia is a condition which has been familiar to farmers and veterinarians for a long time. Its distribution and **symptoms are superficially similar to those of BSE,** and sometimes this has led to confusion. It has been thought that hypomagnesaemia is caused by the failure to absorb the magnesium contained in grass because the faeces which are passed are exceptionally liquid and contain abnormal amounts of the element. This had led to the custom of providing grazing cattle with extra magnesium, and to the injection of magnesium and other salts into cows which have developed tetany. These measures frequently fail to prevent losses, but recently observations have been made which show that cows usually stop passing liquid faeces and are no longer vulnerable to hypomagnesaemia when they graze fields that have been treated with selenium. It has **also been observed that in these circumstances they appear to be resistant to BSE** (3). Rumen bacteria do not behave normally when they are deprived of selenium. In this situation water, together with salts of sodium and magnesium are drawn into the intestine and lost in the faeces. Consequently, the abnormal biochemical conditions develop within affected animals which inhibit mitochondria activity and culminate in tetany. When cattle suffer digestive disorders caused by selenium deficiency the mucus protecting the intestinal epithelium is dissolved, and the opportunity of food particles and fragments of bacterial membrane supplied in meat and bone meal to be absorbed by endocytosis is increased.

BSE

Occasionally **people who have experienced brain surgery later develop CJD**. The simplest explanation of this is that the instruments used during the operations were contaminated with fragments of bacterial membrane and D-amino acids which, when released into the brains which were without D-amino oxidase, stimulate the immunological response that produced spongiform encephalopathy. The moieties that together make up bacterial membranes usually consist of a modified disaccharide to which is attached a short chain of L- and D- amino acids. These moieties are transported to the membrane in combination with undecaprenol phosphate which is similar in structure to dolichol phosphate, the compound used in mammalian glycoproteins synthesis (4). there is no obvious reason why moieties derived from bacterial membranes should not occasionally be

transported by dolichol phosphate and become included within the Pr protein as it is being synthesised. This would distort the structure of the protein but enable the moiety to enter the brain before being detected as being of bacterial origin. The decay of abnormal PrP within the brain with the release of D-amino acids would lead to an immunological response, and presumably to BSE, in a brain that did not contain D-amino oxidase. **It can be concluded that BSE is a metabolic disease which cannot be transmitted either from bovine to bovine or from bovine to man. Its generation requires selenium deficiency, digestive disorder, the presence of a particular type of bacterial membrane and, critically the absence of D-amino oxidase from within the lumen and tissues of the intestine, as well as from the brain**. If the synthesis of D-amino oxidase by mammals depends upon the ability of the deiodinase enzymes to generate sufficient T3, and because the thyroid gland of ruminants is without deiodinase, this explains why bovines are more susceptible to SE than man. It is suggested that those that develop CJD in addition to being selenium deficient have also to be infected by a strain of bacterium with a membrane composed of appropriate moieties. It is reasonable to assume that crops grown in the UK at the present time contain less of selenium than in the past. Heavy metals, including molybdenum complex with selenium (*http://bse.airtime.co.uk/stock.htm).*

References

1. Reilly C (1996) Selenium in Food and Health. Blackie Academic and Professional, p26
2. Ibid, p63 and PP 140-6.
3.Trace element Services Ltd (1997), Abergorlech Rd, Carmarthen. A personal communication.
4. Smith EL et al (1985) Principles of Biochemistry, 7th Edition. McGraw Book Company pp 459, 470 and 471.