Lone voices in the BSE debate

As the BSE hysteria calms, some of the quieter voices are still audible. These are offering alternative explanations for the cause of the epidemic. Though research is still in its infancy, Czech scientist Dr Josef Hlásny firmly believes that scrapie-infected MBM was not the sole culprit.

By Sarah Mellor

Feeding contaminated meat and bone meal to British cattle was the cause of the BSE epidemic which raged through Europe in the 1980's and 90's. Or so we are led to believe. Research into the active agents of TSE's, long tangled protein molecules called prions is still underway. But did the desire to find a cause of BSE and it's human form, new variant Creutzfeld Jakob disease (vCJD) cloud the scientist's judgment? Were they and governments too quick to jump on the prion bandwagon? Indeed, there are some who believe so. In fact, one lone voice believes that the nutritional cause may not be contaminated meat and bone meal (MBM) at all, but that BSE has its roots in a more common nutritional problem.

The basics

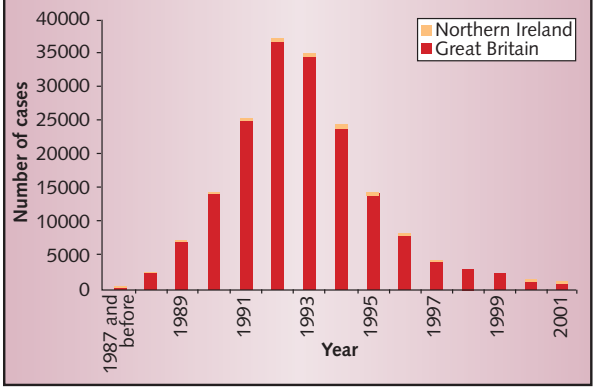
Bovine spongiform encephalopathy (BSE) is a chronic, degenerative disease that affects the central nervous system of cattle. It belongs to a family of diseases known as transmissible spongiform encephalopathies (TSE's), which also includes scrapie (sheep) and Creutzfeld Jakob Disease (humans). The causative agent is yet to be fully characterised. TSE's all share the following characteristics:-

- Prolonged incubation period of months or years
- Progressive debilitating neurological illness which is always fatal
- Electron microscopy of detergent-treated brain tissue reveals the presence of scrapie-associated fibril (SAF)
- pathological changes (including vacuolation and astrocytosis) appear to be confined to the central nervous system
- Transmissible agent elicits no detectable specific immune response in the host. This has inhibited development of a live preclinical animal disagnostic test.

Affected animals may display changes in temperament such as nervousness or aggression, have changes in posture, incoordination and difficulty rising. Despite no loss in appetite, affected animals lose body condition and milk production decreases. The animal's condition continues to deteriorate until it dies or is destroyed.

Since the first case was confirmed in a cow in the UK in September 1984, the disease has spread to native-born cows as far away as Japan. Recent figures are available from the Office International des Epizooties (World Organisation for Animal Health). The epidemic in the UK peaked in the early 1990's (*Figure 1*), and first spread in continental Europe in 1991, where again it appears to have peaked and is now in decline. Though MBM was banned from animal feeds in Europe more than two years ago (January 1st, 2000), the disease still appears, and it is still spreading geographically, if not in incidence (*Figure 2*).

Figure 1. The BSE epidemic in the UK peaked in 1992. Source: OIE (2002)



The consequences

The official report of the BSE Inquiry (2000) commissioned by the British government was a lengthy document, and stated "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. The origin of the disease will probably never be known with certainty."

The scientific argument resulted in the banning of meat and bone meal in animal diets in most parts of the world. The US action appears to have successfully prevented BSE appearing in the national herd at all. But in Britain at least, once the mutated prion was identified as the cause of the problem, all other avenues of investigation were closed down, some would argue, dismissed without sufficient evidence to do so. The British Inquiry took up two of the alternative theories- the organophosphate theory put forward by Mark Purdey, and the autoimmune theory, and dismissed them. These two were not the only alternatives, however (*Box*), and to date, some scientists' experience has been left unheard. Some of these deal with potential nutritional causes as many of BSE's symptoms resemble more common mineral deficiencies or toxicities. In particular, hypomagnesaemia has been implicated as a predisposing factor.

Were we right?

When an article about information sources relating to BSE was published in *FM*'s sister publication *Feed Tech* ("BSE and the Internet" by Dick Ziggers, *FT* Vol. 5, No. 3), a Czech scientist and practising veterinarian was prompted to air his views. Josef Hlásny has been involved in his veterinary capacity,

Figure 2. Spread of BSE across the world. Data as of 22 March 2002. Source OIE. Excludes imported cases

in studies of central nervous neurotoxicities of a nutritional cause, and has recognised a number of threads in common with BSE. For just over a decade, Dr. Hlásny has been involved in research into the use of high-nitrogen forages, and he suspects some of his findings to be relevant to the BSE story.

To date, Dr. Hlásny's work has been published mainly in the Czech language journal *Vyzkum v Chovu Skotu (Rapotin)*. His original letter to *Feed Tech* was some 13 pages long, and so cannot be published in its entirety, but in *BSE - a nutritional neurotoxicity?*, he picks up the story. ●

BSE - a nutritional neurotoxicity?

By Josef Hlásny

The feeding of meat and bone meal to cattle *per se* may not be the main cause of BSE. However, by excessive feeding of MBM, especially in dairy cows, a state of *hyperammonaemia* can be achieved and *hypomagnesaemia* can also be initiated. There are no published references to date in which intake of crude protein (and potassium) high enough to lead to a state of *hyperammonaemia-hypomagnesaemia* during the incubation period of BSE.

Hlásny (2000) reviewed about 200 papers on the central nervous system (CNS) changes associated with BSE, and detected a possibility that these mechanisms have a strong influence on CNS function, especially in ruminants.

Mechanisms of action

Over the past 50 years, yield of many crops has been greatly increased, roughly in proportion to an increase in the use of the application of NPK fertilisers *(eg Table 1)*. Luxury consumption of potassium fertilisers leads to distortion of cation ratios in the herbage: concentrations of Na, Mg and Ca are reduced relative to potassium. Higher N fertilisation increases N concentration in

Can't see the wood for the trees? It is hardly surprising that some of the more viable propositions were not heard, given the sheer number of BSE hypotheses put forward

- BSE derived from scrapie
- BSE is a rare sporadic disease of cattle
- BSE derived from CJD
- Organophosphorus insecticides involved
- Knackers yard greaves involved in transmission
- Bacterial toxicosis causes BSE
- BSE is a lysosomal storage disease
- Horizontal BSE transmission through the eye
- BSE is an autoimmune disease
- BSE transmission is via a *Campylobacter*-like organism
- Alkaloidal glycosidase inhibitors cause changes in the structure of prion proteins
- Smarden spill caused toxic induction of initial BSE outbreak
- Thiosulphide interchange chemistry caused prion protein configuration changes
- Bovine pituitary hormone caused initial spread of disease
- Oligonucleotide involvement in transmission agent
- BSE cases *seen* are vertically transmitted, but a much higher proportion of the herd are asymptomatic
- A range of trace metals (Mg, Cu, Se) may be involved in BSE symptoms and pathology
- BSE controlled by prostaglandin
- BSE is the clinical sign when a chromosomal virus is expressed
- Ubiquitin is important in the formation of TSE agents
- Thiomersal as a preservative in vaccines induces prion protein change
- BSE type illness will result when it is is evolutionary "worthwhile" for a specific protein multiplication
- Retroviral origin of prion disease
- Waste water sludge involved in BSE spread
- Cross-linked dietary proteins involved
- Phonons are involved in prion formation
- MBM use increased in 1980
- D-amino acids alter the prion protein structure
- The endogenous origins of spongiform encephalopathy
- Polypeptide chain folding changes prion protein into an infective form

(Source: http://sparc.airtime.co.uk/bse)

Table 1. The use of NPK fertilisers in the UK, France and Czech Republic since BSE first emerged until 1995

	Nitrogen	P_2O_5	K_2O	Cattle (thousand)*	Sheep (thousand)*
UK 244,872 km²				11,933	29,521
1985-1990	1,556,500	438,250	527,750		
1990	1,582,000	428,000	525,000		
1995	1,412,000	421,000	465,000		
Change 1995/1990 (%)	93	98	89		
France 543,998 km²				21,200	11,900
1985-1990	2,534,300	1,439,050	1,872,375		
1990	2,660,000	1,494,000	1,949,000		
1995	2,308,400	1,030,400	1,373,500		
Change 1995/1990 (%)	89	69	70		
Czechoslovakia 127,900 km²				4,346	874
1985-1990	637,009	494,730	514,655		
1990	704,820	437,000	460,600		
Czech republic 1995	254,000	46,500	40,020		
Slovakia 1995	80,250	16,623	13,800		
Change 1995/1990 (%)	47	14	12		

*1990

the herbage, appears to increase the concentrations of P and K and decreases Mg utilisation by livestock causing hypomagnesaemia. If the P content of young pasture is high, precipitation of insoluble $MgNH_4PO_4.6H_2O$ may begin in the rumen, which also decreases Mg utilisation. Higher N concentration in forages gives higher fatty acid content and decreases the availability of feed Ca and Mg by the formation of insoluble Ca and Mg soaps in the gastrointestinal tract. Highly fertilised young herbage is characterised by a high crude protein content- this is highly degradable causing high rumen ammonia nitrogen concentrations. At elevated blood concentrations, ammonia is toxic to the central nervous system (CNS).

In the liver

In the liver, excess ammonium is generally considered to be eliminated via the ornithine cycle. Blood urea level is closely related to cyclic dietary protein intake, and is inversely proportional to the biological value of dietary protein. The high protein:energy ratio also increases blood urea. The sum of the two processes does not result in accumulation of NH_4 cations in the blood, but in hyperammonaemia because of the liver's diminished capacity to synthesise urea, and to a decrease in glutamine synthetase.

In the fed state, most amino acids (except branched chain AA's) reaching the liver serve as precursors for ureagenesis in the ornithine cycle. Control of this cycle can be either long term, involving synthesis and degradation of enzyme molecules (hours or days), or short-term, via activation or inhibition of existing enzymes (seconds or minutes). Increases in enzyme activity are therefore observed after feeding a protein-rich diet. Together, the two control systems allow large changes in flux through the ornithine cycle at a relatively constant ammonia concentration.

Hepatic encephalopathy

Chronic and acute liver insufficiencies are associated with increased blood ammonia levels. Although there is strong evidence that ammonia is a major neurotoxin, its mechanism of action is still a matter of debate since the clinical signs of chronic hepatic encephalopathy (HE) are reversible. However, in the terminal phase of acute HE, it is irreversible and associated with brain oedema.

The concentrations at which ammonia becomes neurotoxic are somewhat variable. In acute ammonia intoxication encephalopathy, letargy occurs at brain ammonia concentrations exceeding 750μM/kg. Ammonia has major effects on excitory and inhibitory synaptic transmission.

The basic process involved in chemical synaptic nerve transmission is an electrical signal in the presynaptic terminal, which is

changed into a chemical signal that serves as the message. Synaptic potentials are mediated by the amino acids glutamate aspartate, γ-amino butyric acid and glycine.

The classical symptom of magnesium deficiency is tetany. Its biological bases are complex, involving the central and peripheral nervous systems, the neuromuscular junction and the muscle cells. The CSF and electroencephalogram are generally normal in Mg-deficient animals, but these animals are more sensitive to tetany-inducing stimuli such as sound. However, a decrease in CSF Mg concentration occurs in sheep and cattle- together with the associated clinical signs of hypomagnesaemic tetany. In these species this occurs at a CSF Mg concentration of about 0.5mmol/l.

Mg and Ca in neurotoxicity

In the CNS, the Mg ion, Mg^{2+} has two major functions: stabilising synaptic connections and enhancing neurochemical enzyme function. Mg^{2+} has been shown to increase the affinity of various binding proteins including the voltage-dependent Ca^{2+} channel. Because Ca^{2+} ions are normally low in most cells compared with ECF, only a small increase is needed to significantly increase intracellular fluid levels. Free intracellular influx of Ca^{2+} is associated with ischaemic cell death, which may be prevented by competition for ion channels with Mg^{2+}. Mg also enhances intracellular buffering of free calcium ions.

Mg and ammonia in neurodegeneration

In normal to moderate hyperammonaemia, the tendency is towards ammonia formation. Both acute and chronic hyperammonaemia are associated with decreased brain aspartate levels, one of the major CNS neurotransmitters. The most well characterised receptor for amino acid synaptic transmission is the NMDA receptor. Overstimulation of the NMDA receptor results in neurotoxicity and neural injury, involving the influx of Ca^{2+} into neurons.

Studies have demonstrated that Mg can protect against NMDA-induced neurodegeneration, brain injury and convulsions. On the other hand, hypoammonaemia appears to be a cause of primary axon degeneration.

Grass staggers and neurodegeneration

According to the literature, staggers is mostly caused by ingestion of forages that contain tremorgenic mycotoxin, whose mechanisms of action are often incompletely understood, but may involve enhanced release of excitatory amino acid neurotransmitters. However, forages that cause staggers should be considered in the differential diagnosis of hypomagnesaemia. Expression of clinical signs is most directly correlated with CSF Mg levels less than 1.45mg/dl, although normal levels are possible, especially if violent convulsions have been occurring. Commonly grass staggers (grass tetany) usually occurs in lactating cows during winter or spring when grasses are low in Mg, especially when energy intake is less than optimal and during adverse weather conditions. Affected cows may also have been pastured on rapidly growing forage crops that have been over-fertilised with nitrogen and potassium.

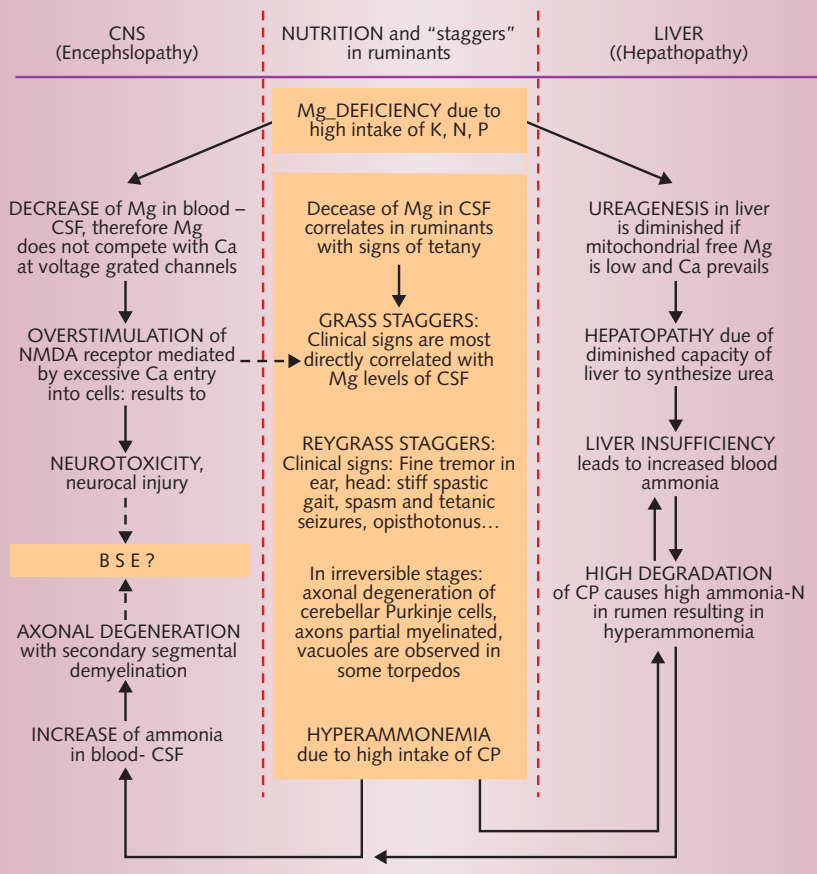
In ryegrass staggers, affected animals appear normal at rest or may have fine tremor in the ear and head. When stimulated, they have a characteristic stiff, spastic gait followed by spasms and titanic seizures, and opisthotonos occurs in severe cases. Recovery may be rapid for perennial rye-, dallies- and bermuda grass staggers if animals are not stressed (within 2 weeks after removal of the toxic forage).

However, in the irreversible stages (longer pasture standing), axonal degeneration of cerebellar Purkinje cells has been found. These lesions in the cerebellar layer often occur in groups with the tendency to be more numerous adjacent to the Purkinje cell layer. Axonal changes (degeneration and vacuoles) are more likely to be found in cattle and sheep the longer the clinical signs have been present.

Generally, it appears that diseases of the nervous system in ruminant are in some way connected with nitrogen and magnesium metabolism. Excessive N and K intake can be associated with hyperammonaemia complicated with subclinical hypomagnesaemia, and neurodegeneration may be involved. These mechanisms should also be incorporated into BSE research. Epidemiological studies to date have shown BSE to occur mainly in countries where significantly higher amounts of NPK fertilisers are applied to forage crops. ●

Dr. Hlásny would like to receive comment from like-minded or interested scientists. A full list of his publications and references is available from Feed Mix on request.

Figure 3. Nervous diseases in ruminants and their nutritional connections. (Hlásny, 2001)



CNS: Central nervous system, CSF: Cerebrospinal fluid, CP: Crude protein, NMDA: "N-Methyl-D-Aspartate" receptor.