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The Pathology and Diagnosis of Bovine Spongiform Encephalopathy

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BSE is one of a family of diseases, the transmissible spongiform encephalopathies(TSEs) which includes other conditions such as scrapie in sheep, and Creutzfeldt Jakob disease and kuru in man. Similar conditions exist in a number of other species. These disease are commonly referred to by several different collective names · TSEs, unconventional slow viruses, prion diseases and infectious cerebral amyloidoses - a fact which reflects the uncertainty which still accompanies their aetiology.

These diseases share a number of common features; they are all chronic, progressive and ultimately fatal neurodegenerations of the CNS which may be acquired, familial or sporadic. They are transmissible, have a prolonged incubation period and provoke no overt immune or inflammatory response. The transmissible agent is yet to be unequivocally identified, but it shows extreme resistance to chemical and physical inactivation, insensitivity to nuclease and consists mainly/solely of an abnormal isoform of a host-encoded glycoprotein(PrP).

The oldest known TSE is sheep scrapie, clear descriptions of which date back some 250 years. Similar diseases of man were first recognized early this century, and it is only within the last 50 years that new examples of naturally occurring TSEs have been described in other animal species. The first case of BSE was diagnosed in 1986.

The principal clinical signs of BSE are apprehension, hyperaesthesia and gait incoordination. In addition a high proportion of animals lose weight or body condition, and milk yield often drops.

Apprehensiveness, altered temperament and behavioural changes are usually the earliest sings seen in closely observed and managed animals. The "hyperaesthesia" may be due to pure hyperaesthesia, hyperresponsiveness or a combination of the two, and is particularly evident in response to touch, particularly on the trunk, the head and the lower hind limbs. This provokes panniculus contractions, tail movements, head tossing and violent kicking. Sudden loud noise or light flashes may precipitate widespread adventitous muscular movements such as fasciculation, tremor and myoclonus, and result in collapse. Such increased reactivity to sudden stimuli appears to result from a combination of an increased alarm reaction, enhanced fear and exaggerated startle reflexes. Increased frequency of behaviour such as licking, sneezing and tooth grinding can be identified periods of during prolonged observation, and specific monitoring techniques can be used to reveal bradycardia, and a marked decrease in the amount of time spent ruminating by affected animals.

It is still not possible, however, to definitively diagnose BSE in the live animal.

The neuropathology of the TSEs comprises vacuolation of neuronal soma and neurites (spongiform change), degeneration and loss of

neurones, a reactive astrocytosis and microgliosis and accumulation, within the CNS, of the protease resistant form of PrP. In some of the animal TSEs this manifests itself as cerebral amyloidosis. These qualitative neuropathological features differ little between species, but the relative prominence of each of the changes and the patterns of their distribution present considerable variation.

In BSE, the most striking and diagnostically significant of these is spongiform change. Vacuoles within neuronal perikarya are also present, and prominent in specific neuroanatomical locations(notably the vestibular nuclear complex in the rostral medulla). The subcellular location and origin of vacuolation is very difficult to determine precisely, but a recent detailed study of natural BSE suggests that the vacuoles may arise in lysosomes, and that the large vacuoles observed by light microscopy could arise as a result of local tissue damage by inappropriately released lysosomal enzymes. Neuronal loss has been shown, but only by the application of morphometric methods. Astrocytic hypertrophy is observed, but is rarely as severe as in scrapie. Cerebral amyloidosis is rarely demonstrable, and would seem to be an unusual feature of BSE.

The immunohistochemical demonstration of accumulated PrP in the nervous system is a diagnostic feature of the TSEs. Since all the antibodies presently available against PrP react with both the normal and disease specific isoforms of the protein, protocols and controls are required which exclude or identify reactivity with the normal isoform. It is always necessary to establish the disease specificity of the variety of configurations and patterns of labelling produced with each antibody. Widespread particulate staining of certain grey matter neuropil is the principal form of disease specific immunolabelling. The topography of immunolabelling corresponds, in general, to the distribution of vacuolar changes in each case. It must be stressed that knowledge of disease specific configurations and distribution of PrP immunolabelling is central to diagnostic application of these methods.

Although histopathological examination for spongiform change has always been the principal laboratory test for confirmation of BSE, the detection of disease-specific 'scrapie associated fibrils'(SAFs) by electron microscopy has been a useful supporting test, particularly where tissue is autolysed or mechanically damaged, or the histopathological result has been equivocal. Biochemically, these SAFs appear to be composed almost entirely of the abnormal protein PrP(Sc27-30kda) which is partially resistant to the proteinase enzymes used in the extraction procedure. The normal host protein PrP(33-35kda) is completely denatured by proteinase enzyme digestion.

When a TSE is experimentally transmitted to mice, it produces a lesion profile(ie neuroanatomical distribution of vacuolar pathology) which is a characteristic 'fingerprint' of that disease, if all experimental conditions are constant. This profile is influenced by several factors, most important of which are agent strain and host genotype. The molecular basis of agent strain is not yet known, but host genotype is determined principally by the PrP gene. Subsequent experimental passage, both intraspecific and interspecific, are known to affect the profile for some, but not all, of the strains of the agent. This method is a major experimental approach for the identification of pathological phenotypes in rodent scrapie and the determination of different strains of the scrapie pathogen.

Lesion profiling has also been applied to define the vacuolar pattern in BSE. The lesion profiles of natural TSEs in animal populations are largely uninterpretable where the host and agent factors show variation, which is the case in natural scrapie. In contrast the lesion profile of BSE is remarkably uniform, mimicking the stereotypy seen in controlled experimental models of rodent

TSEs. From this, and other evidence, it has been proposed that the BSE epidemic has been caused by a single stable cattle-adapted strain of a scrapie-like agent. Repeated profiling of the vacuolar changes in the brains from samples of 100 cattle with BSE has been used to monitor the pathology of the current epidemic in Britain. No evidence of phenotypic changes have been seen in the period 1988~1995, adding further support to the hypothesis that the epidemic has been sustained by a single agent strain.

This uniformity of lesion distribution has been used to validate the current diagnostic procedure of examining a single section of brain(the medulla at the level of the obex) which contains two neuroanatomical 'target' areas - the nucleus of the solitary tract and the nucleus of the spinal tract of the trigeminal nerve - which are consistently affected in BSE.

Despite the detail in which the clinical signs have now been defined, a number(10~15%) of animals slaughtered as BSE suspects do not fulfil the statutory diagnostic criteria of vacuolation of the obex target sites.

Several extensive neurohistological studies have been carried out to determine, where possible, a specific differential diagnosis. One of the most common findings was of encephalitis, in many cases associated with listeriosis. A number of other inflammatory conditions of unknown aetiology have also been observed. In every study 20~25% of cases show white matter vacuolation in the substantia nigra(a feature also observed in approximately 10% of BSE affected animals). The significance of this lesion is unknown. Other conditions such as cerebrocortical necrosis, tumours and congenital cerebellar dysplasias occur at low frequencies (usually less than 10% collectively), and in Scotland a condition known as idiopathic brainstem neuronal chromatolysis has been defined, mainly in older animals from beef herds.

However, the greatest proportion of animals not confirmed as BSE have no significant neurohistological lesions(55~65%). A number of clinical conditions may account for at least some of these animals. Signs associated with ketosis and toxaemia may resemble BSE, and trauma, stress and pain were each evident in some suspects examined in an experimental context. Previously uncharacterised 'behavioural psychoses' have been encountered, and cystic ovarian disease can resemble BSE. Cachexia of unknown origin can be a difficult problem to distinguish from BSE, especially if ecto-parasites, trauma or stress coexist, and transient metabolic/nutritional disorders, due to abrupt alterations in nutrition or environment can cause genuine clinical confusion with BSE. Human factors may also contribute to the reporting of clinically normal cows as suspects, particularly an anxiety to detect cases(ususally on farms with multiple previous cases) and an ignorance of the normal behaviour of cows. In summary, a miscellany of other conditions, some better characterised than others, can be confused clinically with BSE, and effective therapy is often the most appropriate means of differentiating them.

In addition to BSE a number of other conditions can give rise to pathological spongiform change: inherited disease such as branched - chain - keto - acid - decarboxylase deficiency(maple syrup urine disease) and lysosomal disorders; metabolic disturbances such as hepatic encephalopathy and salt intoxication; toxins including ammonium and hexachlorophene, the antibiotic tunicamycin and the anthelmintic closantel; infections such as rabies. Vacuolation may also arise artefactually as a result of autolysis, freezing and thawing of the tissue, inadequate fixation, rapid dehydration, high temperature paraffin wax and prolonged exposure to 70% alcohol during processing. Most of these spongiform changes can be readily distinguished from true BSE vacuolation on careful histological examination (with or without PrP immunostaining).

Epidemiological studies of BSE commenced shortly after its identification in November 1986. These indicated that the first cases occurred in April 1985 and that meat and bone meal, used as a protein supplement in feedstuffs, was the most likely vehicle of a scrapie-like agent. The feeding of ruminant derived protein to ruminants was prohibited in July 1988 and in September 1990 certain specified bovine offals, which from previous research on sheep scrapie were considered likely to certain high titres of the BSE agent, were banned from all animal feed. The first effects of the ruminant to ruminant feed ban were evident in 1991 and 1992 when the incidence in 2 and 3 year old animals was reduced. The national incidence began to decline during 1993. An important aspect of the BSE epidemic is that, whatever the origin, the majority of cases have been due to the recycling of infected cattle tissues via meat and bone meal. Studies and investigations have revealed that there have been problems of accidental cross-contamination of cattle feedstuffs with meat and bone meal in feed mills, producing feed for both ruminants and monogastric animals. In addition, there has been an incomplete compliance with the specified bovine offals ban which should have removed the risks from accidental cross-contamination. As a result, all mammalian derived protein has been prohibited from all farm animal feedstuffs since March 1996.

The evidence is that the incidence of BSE will continue to decline, and will be very low at the beginning of the next millenium.