Histopathological findings in cattle brains collected as negative BSE controls

Samples of central nervous tissue from 1009 clinically normal adult New Zealand cattle were used as negative controls in European trials to validate diagnostic tests for bovine spongiform encephalopathy (BSE). These brains were screened histologically, and no evidence of BSE was detected in them. Incidental hind brain lesions were recorded.


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Materials and methods

Hind brain and anterior spinal cord were collected from 1009 clinically normal New Zealand cattle for use as negative controls in the successful development, in Europe, of tests for the diagnosis of BSE. The protocol for collection and interpretation of findings was approved by the European Commission.

Seven samples were collected from each animal. One sample was formalin fixed, and the rest were frozen.

All of the cattle sampled were at least 4-years-old, and all were killed in one North Island meat works over a period of several months late in 1998. Histological sections were examined from the obex region of the medulla oblongata. Portions of brain containing this site had been fixed in buffered 10% formalin within 30 minutes of slaughter, and embedded in paraffin by routine processing. Duplicate sections separated by approximately 50 microns were cut and stained with haematoxylin and eosin, using Mayer's haematoxylin to maximise contrast between neuropil staining and vacuolar spaces. The sections included the solitary tract nucleus and the spinal tract nucleus of the trigeminal nerve, these being the specific neuroanatomic localities detailed in the European Commission protocol for evaluation of BSE lesions[1] . The cattle were classified histopathologically, according to protocol, as BSE-positive, BSE-negative, or BSE-inconclusive.

The criteria for a positive diagnosis included the presence of characteristic vacuolation of grey matter neuropil and/or neuronal perikaryon, usually bilaterally symmetrical and in targeted neuroanatomical sites - either the solitary tract nucleus or the spinal tract nucleus. A minimal lesion for a positive diagnosis was more than three neuropil vacuoles per histology section.

A negative diagnosis was made in absence of spongiotic lesions in the above sites. An inconclusive diagnosis was applied when a negative diagnosis could not be made confidently or where, for whatever reason, a possible positive diagnosis may have been obscured.

Although not specified in the protocol, an additional category of 'suspicious' was introduced by the New Zealand pathologists for cases where more than three vacuoles in the neuropil occurred unilaterally in one or other of the target sites. These animals were negative for BSE according to the protocol, but this informal category of 'suspicious' was used as a conservative measure to provide a basis for selection of additional cases for Western blot testing.

Unfixed brain from both inconclusive and 'suspicious' samples were sent to the Central Veterinary Laboratory, Weybridge, England for Western blot analysis.

Histological anomalies in addition to those pertinent to BSE screening were recorded.

Results

BSE Screening: Nine hundred and sixty six of the 1009 brains were classified as histologically negative for BSE. Histological changes were inconclusive in 43 brains: 23 because freezing artefact made it impossible to interpret spongiosis in the target areas, six with bilateral loss of critical areas during processing, two because vacuolation was part of a more generalised spongiosis, six classified on very conservative grounds as marginally suspicious according to the specified criteria, and six with occasional vacuolation not considered suspicious according to the specified criteria.

Table 1. Lesions identified in sections at the obex of 1009 clinically normal adult cattle.

Lesion		No. of brains affected
Neuropil vacuolation		
a)	Occasional holes	163 (16.2%)
b)	Numerous holes	180 (17.8%)
c)	Polymicrocavitation	2 (0.2%)
d)	Freezing artefact	23 (2.3%)
Perivascular cuffs		71 (7.0%)
Gliosis		29 (2.9%)
Spheroids		44 (4.4%)
Malacia		2 (0.2%)
Satellitosis		2 (0.2%)
Neuronal somal vacuoles		6 (0.6%)
Total number of lesions		522 (51.7%)

Western blot testing: The 43 brains with inconclusive histology were submitted to the Central Veterinary Laboratory, Weybridge, England for Western blot testing. All were negative.

Additional histopathological changes: Various microscopic abnormalities were identified in addition to those already described. These are summarised in Table 1.

For the purposes of this report the degree of spongiosis was classified as occasional (< 20 vacuoles per obex section) or numerous (> 20 vacuoles per obex section). In most cases the vacuoles ranged in size from 20-50 microns, were round or ovoid and devoid of content, and most were distributed widely and usually randomly throughout the neuropil. In all cases where vacuoles were identified within the target areas for BSE examination, a similar concentration of vacuoles were demonstrable elsewhere in the section.

Vacuoles within the neuronal soma were identified in six brains. The sites affected included the vagal, ambiguus, trigeminal, solitary, and olivary nuclei, and one in the reticular formation.

Multiple lesions were relatively common, with perivascular cuffs and vacuoles occurring in 27 sections, gliosis and vacuoles in 10 sections, and gliosis and perivascular cuffing in 11 sections.

Discussion

The incidental lesions recorded here are similar to those already documented as incidental changes in clinically normal sheep and goats[2][3], but these appear to be the first published findings in cattle. The present work documents changes at only one site in the brain, but reports for other species, along with anecdotal information, suggest that the changes were likely to have also occurred at other locations in the CNS of these cattle. It is critical that the significance of any particular lesion reported here be assessed according to its neuroanatomical location in respect of clinical signs , the age and nature of the change, and the presence of other other related pathological changes. Having access to only one site in the brain seriously limits the clinico-pathological interpretations that can be applied to these findings.

Vacuolation of the neuropil was by far the most common change observed in the present series. This change has assumed an exaggerated importance because of its association with the transmissable spongiform encephalopathies[4] , but it is important for pathologists to recognise that diffuse spongiosis can be an incidental finding.

The cause of vacuolation in most of the present cases was unknown. Spongiotic change may result from a diverse range of cellular insults, and can derive from spaces within the processes of the neuropil, myelin sheaths, or swelling of oligodendrocyte or astrocyte cytoplasm. Ultrastructural examination is often needed in order to identify the source of the change observed by light microscopy.

In myelinated fibre tracts, oedema-induced vacuolation is a common abnormality in which fluid accumulates as vacuoles in the inter-period region of the myelin sheath. In the present series, severe polymicrocavitation of this type was present in two brains, the change being distributed throughout the myelinated nerve fibre bundles of both grey and white matter. This lesion is commonly associated with hepatic disease and (presumed) hyperammonaemia[5]. Although the animals in this trial were clinically normal, the region from which they came had been seriously affected by facial eczema in the previous season, and residual damage to the neuraxis referable to resolved sporidesmin toxicosis may have explained the change in these cases. The other milder expressions of vacuolar change seen in this series of brains could also have been induced by sporidesmin toxicosis. Similar scattered holes in white matter have been reported as artefact in tissue exposed to prolonged immersion in 70% alcohol during histological preparation[6], but this could not have occurred in these cases.

Neuronal vacuolation is a well recognised incidental finding in the obex region in sheep[3], and in the red nucleus of cattle[7]. The present series indicates cattle may also have incidental neuronal vacuolation in the obex region.

Spheroids, which represent distended nerve processes containing disorganised neurotubules and fibres, are a relatively common finding, occurring frequently in the cuneate nuclei of the dorsolateral medulla oblongata. This site, with its synaptic connections to long ascending nerve processes of the spinal cord, is a common location for such degenerative changes in a number of domestic animal species[2][8].

Perivascular cuffs are a hallmark of central nervous system inflammation and immunological response. In the present series, all except one lesion were of mononuclear leucocytic composition. The extent of the lesions varied considerably. Some were widespread and affected many blood vessels, but more commonly they were restricted to single blood vessels only.

Neuroglial proliferation is a common and non-specific response of these cells to many forms of injury and it can follow inflammation, ischaemia, trauma, and toxic injury. In several of the brains, gliosis was observed in association with other changes, notably perivascular cuffing and satellitosis, suggesting an active process rather than the more common, and presumed quiescent, post-inflammatory stigma.

References

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