The Topographic Distribution Pattern of Vacuolation in the Central Nervous System of Cattle Infected Orally with Bovine Spongiform Encephalopathy

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Abstract

Bovine spongiform encephalopathy (BSE) is a scrapie-like disease of cattle first recognized in the United Kingdom in 1986. The predominant pathology is a vacuolar degeneration of the central nervous system. In naturally affected cattle the severity and distribution of these vacuolar changes present an almost invariable pattern.

The source of infection responsible for the current epidemic is considered to be commercially processed feed contaminated with a scrapie-like pathogen. A foodborne source of infection is also considered the likely origin of scrapie-like disease in other mammalian species, including the domestic cat and several species of exotic bovids and felids in zoological collections, which have occurred contemporaneously with the BSE epidemic.

In common with other scrapie-like diseases BSE has been transmitted experimentally to several other species. The transmission of BSE to cattle by the oral dosing of calves with BSE-affected brain stem homogenate has been reported previously. Histopathological examinations of the brain based on the lesion profile system, developed originally for the characterization of scrapie in laboratory mice, were used to examine the distribution and severity of vacuolar changes in these cattle. The results were compared with the vacuolar profile in naturally affected cattle. The distribution of vacuolar changes in cattle dosed orally with BSE closely resembles that observed in naturally affected cattle providing experimental evidence that this route closely simulates natural infection and subsequent neural pathogenesis.

Keywords: *Bovine spongiform encephalopathy, lesion profile, oral challenge, vacuolation.*

Introduction

Bovine spongiform encephalopathy (BSE) is a novel disease of cattle which was first recognized and defined in the United Kingdom in 1986 in laboratories of the State Veterinary Service in the course of routine diagnostic neuropathological examinations of food animal diseases (Wells, Scott, Johnson, Gunning, Hancock, Jeffrey, Dawson & Bradley 1987). The characteristic neuropathology and the experimental transmissibility of BSE to other species (Dawson, Wells, Parker, Francis, Scott, Hawkins, Martin, Simmons & Austin 1994, Fraser & Foster 1994) indicate that BSE is caused by a scrapie-like infectious agent.

Epidemiological investigations have shown that the vehicle of infection was meat-and-bone meal, incorporated into concentrate feedstuffs as a protein-rich supplement (Wilesmith, Wells, Cranwell & Ryan 1988). BSE was probably started by infection of cattle with scrapie, but the subsequent course of the epidemic was driven by the recycling of infected cattle tissues within the cattle population via feedstuffs (Wilesmith 1994). A foodborne source of infection with BSE is also considered the likely origin of scrapie-like diseases in certain other mammalian species, including the domestic cat and several exotic bovids and felids in zoological collections, which have occurred contemporaneously with the BSE epidemic (Wilesmith 1993).

In common with other scrapie-like diseases, BSE has a distinctive central nervous system pathology principally characterized by bilaterally symmetrical vacuolation involving neuronal processes and soma. A method called lesion profiling, originally described in experimental models of scrapie in mice (Fraser & Dickinson 1968), is used to quantify the neuropathology of TSEs. The method is a highly reproducible scoring approach to the severity of vacuolar changes according to neuroanatomical location when carried out in the late stages of clinical disease. The lesion profile is dependent on several variables, mainly the 'strain' of the scrapie agent and the mouse genotype, with regard particularly to the *Sinc* (or PrP) gene which controls incubation period in mouse scrapie. Provided all other variables are kept constant, as is possible in inbred mice, the profile provides a unique identification of the 'strain'

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of agent. When the latter is a constant other factors influencing the profile, including route of inoculation (Fraser 1976) can be investigated.

Adaptations of the lesion profiling method have been used to study the vacuolar pattern in BSE (Wells, Hawkins, Hadlow & Spencer 1992, Simmons, Harris, Jeffrey, Meek, Blamire & Wells 1996), Feline Spongiform Encephalopathy (FSE) of domestic cats (Wells, Hawkins, Cunningham, Blamire, Wilesmith, Sayers & Harris 1994) and Chronic Wasting Disease of mule deer and elk (Williams & Young 1993). Lesion profile results of BSE have presented an almost invariable pattern of vacuolar changes suggesting, by analogy with lesion profiling studies of mouse scrapie, that the BSE epidemic is sustained by a single major strain of agent (Wells, Hawkins, Hadlow & Spencer 1992).

Here we describe a study to determine the lesion profile of cattle terminally affected after oral challenge with BSE and to compare it with the lesion profile obtained in naturally affected cattle.

Materials and Methods

The cattle brains used in this study were taken from two experiments. In the first, designed to examine the pathogenesis of BSE (Wells *et al.*, in press), a group of 30 calves were dosed orally, at four months of age, with 100g of a homogenate of pooled brain stems from cases of BSE, confirmed in 1991. Groups of these cattle were killed at intervals through to 40 months post inoculation (p.i.). Only a small proportion of those killed developed disease, from 35 months p.i. In the second experiment a further group of 40 calves, selected at the same time and from the same sources as those above, were divided into four groups of ten animals. Each group was then challenged orally as in the first experiment, but with either a single 1g, 10g or 100g dose, or a 100g dose on three successive days (Dawson *et al.*, 1994).

Ten clinically and histopatholgoically confirmed cases of BSE of mixed breeds of cattle from these experiments were selected at random from animals which had received either a single 100g dose (n = 6) or the multiple 100g dose (n = 4). The brains, previously fixed in 10% formal saline, were cut and sampled at seven levels to ensure representation of all major brain regions. Lesion profiling was carried out on haematoxylin and eosin stained sections by scoring 17 neuroanatomically distinct sites (Table 1) according to the method previously detailed by Simmons *et al.*, (1996). The sites scored were considered to be representative of different intensities of vacuolar pathology in the lesion profile of naturally occurring BSE. To ensure uniformity of scoring the observers who carried out the scoring of each selected area in the previous study (Simmons *et al.*, 1996) were the same in this study and inter-observer variation was controlled as described previously. The mean score for each neuroanatomical area was calculated, and plotted against the area 'code number' to produce the lesion profile. Equivalent data from natural field cases of BSE studied over three annual periods (Simmons, Harris, Meek, Blamire, Jeffrey & Wells - in press) were compared statistically with that of the orally challenged cattle.

Results

The mean (±sd) scores for each neuroanatomical area are shown in Table 1 and the lesion profile is plotted in Fig. 1. The equivalent lesion profile from natural field cases of BSE studied over three annual periods (Simmons *et al.*, in press) is shown for comparison (Fig. 1).

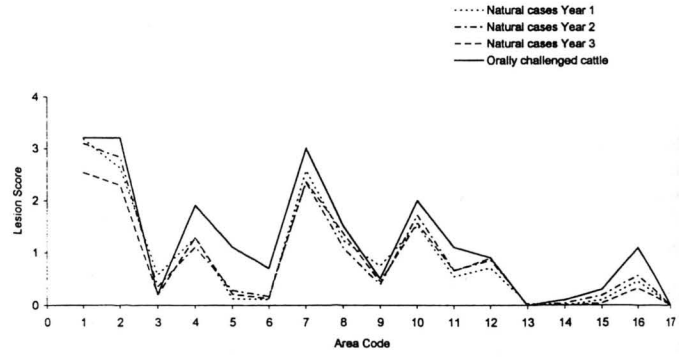

Figure 1.

Table 1. Area code and mean vacuolar score of severity of vacuolation in each neuroanatomical area

Area code	Neuroanatomical area	Lesion score (± SD)
1	Nucleus of the solitary tract	3.20 (±1.03)
2	Nucleus of the spinal tract of V	3.20 (±0.65)
3	Hypoglossal nucleus	0.20 (±0.42)
4	Vestibular nuclear complex	1.90 (±1.10)
5	Cochlear nucleus	1.10 (±0.74)
6	Cerebellar vermis	0.70 (±0.48)
7	Central grey matter	3.00 (±1.05)
8	Rostral colliculus	1.50 (±0.85)
9	Medial geniculate nucleus	0.50 (±0.71)
10	Hypothalamus	2.00 (±0.94)
11	Nucleus dorsomedialis thalami	1.10 (±0.88)
12	Nucleus ventralis lateralis thalami	0.90 (±0.74)
13	Frontal cortex	0.00 (±0.00)
14	Septal nuclei	0.10 (±0.32)
15	Caudate nucleus	0.30 (±0.48)
16	Putamen	1.10 (±0.57)
17	Claustrum	0.00 (±0.00)

The shape of the profile obtained from orally inoculated cattle is closely similar to that obtained from naturally occurring cases of BSE. In fourteen of the seventeen neuroanatomical locations the mean vacuolation score was significantly different ($p < 0.05$ - $p < 0.001$) from the previous three separate studies of the natural disease cases.

Discussion

The main finding from this study is the similarity between the lesion profile of the experimentally orally challenged cattle and those of the natural cases. This observation is entirely consistent with rules controlling the lesion profile in inbred mouse models of scrapie (Fraser 1976, Bruce, McConnell, Fraser & Dickinson 1991) and is in accord with the view that the BSE epidemic has been sustained by a single major cattle adapted strain of scrapie-like agent which is stable after recycling in cattle via rendering (Kimberlin 1993, Simmons *et al.*, 1996) or, as in the present studies, by experimental passage. That the strain of agent is singular and remarkably stable through the epidemic is also indicated by studies of at least 7 isolates of BSE which on primary passage in a panel of inbred mouse strains produce identical phenotypic characteristics (Bruce, Chree, McConnell, Foster, Pearson & Fraser 1994). Stability of the BSE profile is also consistent with the apparent lack of variability of host genetic factors which could impinge on the disease phenotype in cattle (Wilesmith *et al.*, 1988, Goldmann, Hunter, Martin, Dawson & Hope 1991, Hunter, Goldmann, Smith & Hope 1994). The lesion profile in mouse scrapie models is not affected by dose of inoculum. This would apparently be true also for the profile in cattle with BSE since, from epidemiological evidence, natural infection is considered to result from low dose exposure (Kimberlin & Wilesmith 1994), whereas in the present study high doses were used.

Differences in the height of the profile in this study, at least in some neuroanatomical locations, compared to the natural disease profiles, is likely to be related to factors other than the major variables of strain of agent or PrP genotype of the host. However, variation in the profile between breeds of cattle, which may be related to differences in PrP genotype, have been found (G.A.H. Wells - unpublished). Although route of inoculation is an important minor variable in determining the lesion profile in inbred mouse models of scrapie (Fraser 1976) significant differences are seen only between the intracerebral and peripheral routes, the latter including intraperitoneal, subcutaneous and intravenous routes (Kimberlin, Cole & Walker 1987). Furthermore it is unlikely that experimental dosing by mouth and ingestion via feed are substantially different in terms of route of infection in cattle.

Differences in the height (Fraser & Dickinson 1968) and differences both in height and shape of the profile (Cole & Kimberlin 1985) have been shown to occur during the late incubation period and through the clinical disease stage in mouse models of scrapie. Since severity of vacuolar change in the brains of cattle with BSE increases with clinical duration (Wells *et al.*, 1994) this could also explain differences in profile height in the present study from that experienced in natural disease. Furthermore, slight variation in the shape of the profile is seen when the data of small subsets of natural cases of BSE are compared (M.M. Simmons - unpublished). In view of the small experimental sample size here, this too may have contributed to minor variations in the profile from that of the natural disease data.

Nevertheless, the close similarity of the lesion profile of the orally challenged cattle with those of the natural cases provides experimental evidence that the oral route closely simulates natural infection and subsequent neural pathogenesis.

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Abstracts

Bovine retained placenta: aetiology, pathogenesis and economic loss

R. A. Laven, A. R. Peters
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The literature on the effects and causes of retained placenta in the cow is reviewed. On a herd basis the condition can adversely affect milk yield and fertility, but on an individual cow basis the effects are unpredictable. The aetiology of retained placenta has been extensively studied and many causal factors have been implicated, but little is known of how many of them cause the condition. As a result its prevention and prediction is uncertain, primarily because of the lack of knowledge of the normal process of placental release. Vascular changes and uterine contractions play a role in placental release, but current opinion suggests that the primary cause of retained placenta is the retention of the feto-maternal union. Release only occurs after a process of maturation, which involves hormonal and structural changes. The factors which are thought to influence these changes, and thus cause the condition, are discussed.

Incidence of production diseases and other health problems in a group of dairy herds in England

R. J. Esslemont, M. A. Kossaibati
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The incidence of major production diseases and other health problems was investigated in 90 Friesian/Holstein dairy herds in England (average size 152 cows) for cows calving during 12 months in 1992-1993. The mean incidence of mastitis was 33.2 cases per 100 cows, and it affected 20.6 percent of the herd with 1.6 cases for each affected cow. On average, 17.4 percent of the cows suffered from lameness, with 1.4 cases per affected cow and a total of 24.0 cases per 100 cows. Cows treated for oestrus-not-observed totalled 33.6 percent, with 46.4 treatments per 100 cows. The incidence of milk fever was 7.7 cases per 100 cows. Retained fetal membranes affected 3.6 percent of cows. Vulval discharge affected 15 percent of the average herd with 1.4 treatments per affected cow and a total of 21.2 treatments per 100 cows. The mean incidence of twinning was 4.1 percent. Calf mortality claimed 7.8 calves per 100 calves born. The average number of cows given aid at calving was 8.7 per 100 cows calving.