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Transmission Studies of BSE in Sheep

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Introduction

The origin of BSE in cattle is still considered to have been scrapie contamination of meat and bone meal in compounded feedstuffs (Wilesmith and others 1988). There are similarities between BSE and scrapie based on neuropathological and biochemical studies. The possibility that BSE may have transmitted to sheep has been considered and for this reason it was decided to transmit BSE into genetically defined sheep and goats, and observe the biological response in these species. For comparative purposes, a natural scrapie isolate was also used for transmission to sheep and goats.

Two routes of transmission were chosen: a. intracerebral (ic) injection because it is known to be one of the most efficient routes of injection, especially in goats, and b. oral challenge because this route mimics the ingestion of contaminated feedstuffs which propagated the BSE epidemic.

The objectives of this study were to observe challenged animals for the appearance of clinical signs of disease and to recover tissues (brain and spleen) for diagnosis and/or subsequent transmission. It was also important to compare the relative efficiency of infection between the two routes of challenge. Traditionally the oral route has shown itself to be much less efficient than other routes, at least in rodents (Kimberlin and Walker 1989).

Methods

Sheep were chosen from the Neuropathogenesis Unit's closed flock of Cheviots which has been selectively bred over many years as two genetically discrete lines. The lines have been designated "positive" and "negative" referring to their relative susceptibilities to the SSBP/1 experimental sheep scrapie isolate following subcutaneous inoculation (Dickinson and Outram 1988). Susceptibility to scrapie in these sheep is related to polymorphisms in the PrP or prion protein gene (Goldmann and others 1994). While the "positive" line produces a few cases of natural scrapie each year, the "negative" line has never yielded a case in thirty five years (Hunter and others 1996). The goat herd also remains free of natural scrapie. Sheep were retrospectively genotyped at three PrP amino acid codons (136, 154 and 171) using polymerase chain reaction (PCR) and sequencing of the PrP gene (Goldmann and others 1994).

Pooled brain homogenates from four BSE-affected cows were injected ic (0.5 ml, 10^{-1} dilution) or administered orally (50mls, 10^{-2} dilution) to sheep and goats (aged at least three months). The sheep and goats were segregated from other livestock in a paddock bounded by double fencing with a 2 m gap. They were kept at pasture throughout the year and during the winter months received supplementary hay. Commercially compounded feed supplements were never fed to sheep, but the goats received approximately 0.5kg/head/day of compound feed purchased after the meat and bone meal exclusion order of July 1988.

For the natural scrapie transmission experiment sheep were also selected from the "positive" and "negative" lines and genotyped. Brain homogenate from a half-bred natural scrapie sheep ($VA_{136}/RR_{154}/QQ_{171}$) was injected ic (0.5 ml, 10^{-1} dilution) or administered orally (50 mls, 10^{-2} dilution). Challenged animals were pastured with other experimental sheep.

Brain and spleen were collected aseptically from clinically suspect cases in both of the transmission experiments. Full pathological examination of brain was performed routinely on all animals whether clinically affected or killed/died for other reasons. Immunocytochemistry and western blotting for the PrP protein were also undertaken if clinical and histological diagnosis of disease were compromised.

Results

The results of this study are divided into two sections, the first refers to sheep which had been challenged with BSE, and the second to sheep which had been challenged with a natural scrapie isolate.

BSE transmission experiments

For intracerebrally injected "negative" line sheep ie. those sheep which are AA at codon 136, five out of six challenged animals developed disease. Two sheep typed as homozygotes ($AA_{136}/RR_{171}/\mathbf{QQ}_{171}$) had shorter incubation periods ($\leq$ 500 days) than the three heterozygotes ($AA_{136}/RR_{154}/\mathbf{QR}171$ - $\geq$ 1600 days). Clinical signs of disease in three sheep lasted between 2 and 5 days when they showed a rapid progression of ataxia leading to recumbency and in one case death. There was no evidence of pruritus.

The two other ic challenged sheep had difficulty walking and seemed to present as arthritis. Both sheep exhibited their arthritis-like clinical signs for at least two weeks prior to death, which could have masked signs more typically attributable to infection with BSE. Vacuolar degeneration was observed in the thalamus/hypothalamus and to a lesser extent in the mid-brain, of 4 from 5 sheep. One sheep died and diagnosis by light microscope was impossible, however, Western blotting showed positive for PrP^{SC}.

Only one case of disease manifested in the "negative" line in a $AA_{136}/RR_{154}/QQ_{174}$ genotype sheep after oral challenge at 734 days post dosing. Clinical disease progressed very rapidly over three days and histological assessment of brain sections from this oral case demonstrated vacuolation in some of the medullary nuclei and in the mid-brain/thalamus. Brain and spleen from a 440 day ic case and the 734 day oral case were used for transmission studies in mice to detect BSE infectivity (Foster and others 1996).

Three goats injected ic with BSE developed disease at between 500 and 600 days. Of three challenged orally, only two developed disease, while the third died suddenly with no clinical signs and negative histopathology. The three ic injected goats and one dosed orally quickly developed a pronounced ataxia and had to be culled within six days of observation. The other oral case became lethargic and lost weight over a period of three weeks prior to sacrifice with no evidence of ataxia or pruritus. The ic injected goats showed pronounced vacuolation in the thalamus/hypothalamus. Vacuolation also occurred in some brain stem nuclei, but was less intense and did not appear in every case. One orally dosed goat showed only low levels of vacuolation in the mid-brain and thalamus, while the other had more extensive lesioning, especially in the diencephalon

From the ic challenged "positive" line sheep ie. those sheep with genotypes predominantly of $VV_{136}/RR_{154}/QQ_{171}$, $VA_{136}/RR_{154}/QQ_{171}$ or $VA_{136}/RR_{154}/QR_{171}$, three possible cases developed. When sheep from this line were challenged orally, another three possible cases resulted. The range of incubation periods in these six sheep was 2 to 5 years. One "positive" line ic injected sheep died suddenly having shown signs of incoordination 2 to 3 days previously, but with no pruritus. The other two developed ataxia and were sacrificed in less than 7 days of signs

first appearing. One of the orally dosed "positive" line sheep died before a clinical assessment could be determined, while the other two gradually lost condition over approximately six weeks without any obvious signs of incoordination or scratching.

Two ic challenged sheep from the "positive" line showed sparse vacuolation of the medullary nuclei and diencephalon, but had more pronounced leisioning in the mid-brain. The other ic case demonstrated slight autolysis caused by sudden death, but had very mild vacuolation in the raphe and cuneate nuclei, however, Western blotting of brain tissue proved negative.

Two of the orally dosed sheep had vacuolation, mostly confined to the brain stem with very little or none through the diencephalon and cortex. This was similar to what is normally observed with natural scrapie. The other case died precluding a satisfactory histological diagnosis, although some very mild lesions were present and negative Western blotting.

Natural scrapie isolate transmission experiments

Transmission of a natural scrapie isolate to sheep and goats showed that ic inoculation was a more efficient route of infection than oral dosing. For example, in goats all three ic challenged animals developed disease within 517 days of injection. They exhibited signs of ataxia for up to three weeks prior to sacrifice, before becoming recumbent. Pruritus was only observed as an incipient condition in one goat. The three orally drenched goats survive at the time of writing 2200 days post challenge.

In sheep, the ic inoculated "positive" line produced four cases of transmission within 820 days of challenge, three with the PrP genotype $VA_{136}/RR_{154}/QR_{171}$ and the fourth $VA_{136}/RR_{154}/QQ_{171}$. They developed signs of unsteadiness and had to be sacrificed or died within five days of first being observed, while two became recumbent. From the orally dosed "positive" line, there were two prospective transmission cases. From the ic injected "negative" line sheep two experimental cases developed at 2 - 3 years post-inoculation with a rapid onset ataxia over a few days. Oral challenging of "negative" line sheep failed to generate infection in any of the experimental animals.

Vacuolation in ic challenged sheep and two of the goats was mostly confined, although not exclusively, to a moderate lesion of the diencephalon, which is characteristic for this route of challenge. One goat had much milder vacuolation in this area. Oral challenge of a "positive" line sheep only produced two or three vacuolar lesions in the brain stem. In one ic challenged "positive" line sheep and an other oral case, autolysed brain compromised an histological diagnosis, however, Western blotting was positive for PrP^{SC} in all these three cases. There did not appear to be obvious differences in vacuolation which could be attributed to specific PrP genotypes.

Discussion

This study has demonstrated that BSE can be transmitted to sheep and goats following either intracerebral or oral challenge (Foster and others 1993). It was shown that the intracerebral route of infection was the more efficient of the two, as was also observed in natural scrapie isolate transmissions. Both sets of data are based predominantly on the infection of sheep of defined PrP genotypes and goats, which do not contract natural scrapie on Skedsbush experimental farm.

The incidence and length of incubation period in BSE challenged sheep is governed by the presence of the amino acid glutamine (Q) at codon 171 of the ovine PrP gene, the homozygous form $AA_{136}/RR_{154}/QQ_{171}$ having a much shorter disease period than the heterozygotes $AA_{136}/RR_{154}/QR_{171}$. It is important to note that the genotype $VA_{136}/RR_{154}/QR_{171}$ has never supported a case of natural scrapie (Hunter and others 1996) and transmission in these

sheep produce incubation periods very similar to those from transmission cases with the genotype $AA_{136}/RR_{154}/QR_{171}$, which confirms the partial dominance of glutamine$_{171}$ in defining the pattern of BSE disease in these sheep.

The incidence of transmission with the natural scrapie isolate does not appear to be under the same genetic influence of glutamine$_{171}$ as occurred with BSE transmission. This was made clear because with intracerebral challenge in the "positive" line, there were four transmission cases in $VA_{136}/RR_{154}/QR_{171}$ or QQ_{171} genotypes of incubations of less than 1000 days.

There appears to be other differences in the transmission properties between BSE and this natural scrapie isolate, which relate to disease incidence. For example, the oral transmission of BSE to goats produced two cases which contrasts with the less efficient transmission of the natural isolate, which failed to generate any cases. In sheep, although only one case has occurred in the "negative" line following oral challenge with BSE, none were produced following oral challenge with the natural isolate. The intracerebral inoculation of "negative" line sheep with BSE also produced a greater incidence of disease than their counterparts challenged by a similar route with the natural scrapie isolate.

The only circumstance in which infection with the natural isolate produces an higher incidence of disease compared to BSE, is in intracerebrally (and possibly orally) challenged "positive" line sheep. Notwithstanding the possibility of indigenous natural scrapie in some of these sheep, there are still sufficient numbers of transmission cases with PrP genotypes which preclude the natural disease developing i.e. those typed as $VA_{136}/RR_{154}/QR_{171}$.

As an extension to this study, it has been possible to recover BSE by passage in mice from brain and spleen taken from "negative" line sheep infected with BSE by ic and oral challenge (Foster and others 1996). The close similarity of incubation periods and pathology from the passage of these tissues in mice to those seen in direct BSE transmissions from cattle to mice suggests that passaging BSE in sheep does not alter its biological properties (Bruce and others 1994). In fact, because it has been possible to isolate BSE infectivity from ovine spleens, when this proved impossible from the spleens of naturally infected BSE cows (Fraser and Foster 1993), experimentally-induced BSE in sheep appears to behave more like the natural disease of scrapie. Whether this putative similarity to natural scrapie extends to the possibility of maternal transmission of experimentally-induced BSE in sheep, has still to be elucidated.

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