EXPERIMENTAL TRANSMISSION OF BSE TO SHEEP

DRAFT PAPER FOR PUBLICATION

"Detection of BSE in brain and spleen of experimentally infected sheep" by Foster, Bruce, McConnel, Chree and Fraser.

The attached paper has been passed to us for comment prior to publication. Minor changes will be made as a consequence of comments received, but these will not affect the detail of the paper. It is relevant to discussion on 8 March on the subject of BSE in sheep.

D Matthews
Detection of BSE in brain and spleen of experimentally infected sheep

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Bovine Spongiform Encephalopathy (BSE) has been transmitted previously to mice by the inoculation of brain homogenates from BSE-infected cattle. In such experiments BSE is characterised by a specific pattern of incubation periods and neuropathology in panels of inbred mouse strains (Fraser et al 1992; Bruce et al 1994). These characteristics can be used to identify the BSE strain of agent in transmissions from other species e.g. domestic cat (Fraser et al 1994; Bruce et al 1994). Attempted transmissions from non-neural tissues of BSE cattle, including spleen, have so far been unsuccessful (Fraser 1994).

Scrapie infectivity has been detected in the spleens of some animal species following natural or experimental challenge. Studies on the pathogenesis of natural scrapie in sheep (Hadlow 1982) have shown that infectivity is present in the spleens of infected animals. This is also true for goat
or sheep spleen following experimental infection with scrapie (Pattison 1960; Hadlow 1974; Stamp et al 1959).

In mice high titres of infectivity were demonstrated in the spleen following intraperitoneal, intracerebral or subcutaneous challenge with scrapie (Ecklund et al 1967; Dickinson and Fraser 1969; Kimberlin 1989). It is now recognised that the spleen is important, but not essential, for the successful initiation of scrapie infection in mice, prior to neuro-invasion of the brain following peripheral challenge (Kimberlin 1980, 1983; Fraser 1992).

The present study shows that BSE infection is detectable in the brain and spleen of genetically defined sheep following their experimental challenge by either the intracerebral or oral route. Sheep were chosen from the NPU 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 injection (Dickinson and Outram 1988). Susceptibility to scrapie in these sheep is related to polymorphisms in the PrP or prion protein gene (Goldmann et al 1994). Natural scrapie does not occur in the "negative" line of our Cheviot flock.

A previous study has shown that "negative" line sheep can be experimentally infected with BSE by intracerebral (ic) or oral challenge (Foster et al 1993). Transmission to these sheep was achieved following ic injection of 0.5 mls of a 10% homogenate prepared from a pool of 4 BSE cow brains (BSE 1 - 4 in Table 1). This resulted in 5/6 sheep developing disease
with incubation periods of between 440 and 2353 days post-injection, dependent on their PrP genotypes (Goldmann et al 1994; Goldmann, in preparation). Oral challenge of 6 "negative" line sheep produced one case with an incubation period of 734 days following the administration of 50 ml of 1% of the same brain homogenate (equivalent to 0.5 gms of brain per sheep).

Brain and spleen were recovered aseptically from this single orally infected sheep and from an ic injected sheep with an incubation period of 440 days. Both sheep had the same PrP genotype ie. Alanine/Alanine

Glutamine/Glutamine

(Goldmann et al 1994, subscripts refer to codons in the PrP gene sequence).

Tissue homogenates were injected by a combination of ic and intraperitoneal routes into panels of mouse strains (approximately 24 mice per strain) by a standard protocol used previously to compare the transmission properties of BSE, scrapie and spongiform encephalopathies from other sources (Fraser et al 1992). Incubation periods were measured up to a standard end-point when the mice were showing unequivocal clinical signs of neurological disease (Dickinson et al 1968). The incidence of disease in each strain of mice was in excess of 50%, except where incubation end-point approached normal lifespan. The distribution of vacuolar degeneration in the brains of infected mice was assessed to construct "lesion profiles" (Fig. 1) as described previously (Fraser and Dickinson 1968).

Transmissions from the brains of both BSE-infected sheep gave incubation periods (Table 1) and pathology (Fig. 1) in
mice that were closely similar to those seen in direct BSE transmissions from cattle to mice (Bruce et al 1994; Foster et al 1994).

Infectivity was also detected in the spleens of both sheep, although the slightly longer incubation periods following spleen transmission compared to brain from the same animal may indicate lower levels of infectivity in the spleen. The patterns of incubation periods (Table 1) and pathology (Fig. 1) in the panel of mouse strains were again closely similar to those seen with BSE from cattle. This confirms that the infection recovered from the spleens of these experimentally challenged sheep was BSE and not a hypothetical subclinical, natural scrapie infection.

As a comparison, transmissions from brain and spleen from a Greyface sheep with natural scrapie, which had a PrP genotype of Valine/Alanine121: Glutamine/Glutamine121, gave incubation periods (Table 1) and lesion profiles (Fig. 1) that clearly differed from those of cow or sheep BSE transmissions. Attempted transmissions using brain and spleen from unchallenged "negative" line sheep have remained negative at 465 days post-injection with no clinical cases in any injected mice. This is greater than 100 days beyond the incubation periods for spleen transmission to R111 mice from either of the BSE infected sheep.

Our studies of experimental BSE in sheep have indicated that replication or accumulation of the infectious agent occurs in brain and spleen, irrespective of route of challenge, and that the isolate has maintained remarkable stability as demonstrated by transmission to inbred mice. The
significant levels of infectivity found in the spleens of these sheep show that the pathogenesis of BSE in this species is more akin to that expected for natural and experimental scrapie in sheep, and differs from that of BSE in cattle.
References


Dickinson A.G., Meikle V.M.H. and Fraser H. (1968) Journal of Comparative Pathology 78, 293


Eklund C.M., Kennedy R.C. and Hadlow W.J. (1967) Journal of Infectious Diseases 117, 15

Foster J., Hope J. and Fraser H. (1993) Veterinary Record 133, 339


Fraser H. and Dickinson A.G. (1968) Journal of Comparative Pathology 78, 301


Pattison I.H. and Millson G.C. (1960) Journal of Comparative Pathology 70, 182

Table 1. Incubation periods (days ± SEM) in mice following transmission of brain and spleen from two clinically affected "negative" line sheep, one of which had been challenged by intracerebral (ic) inoculation and the other by orally drenching with BSE homogenates. These are compared with pooled transmission data from four BSE cows and one natural scrapie sheep.

<table>
<thead>
<tr>
<th>Source of infection</th>
<th>Mouse Strain</th>
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<tbody>
<tr>
<td></td>
<td>RIII</td>
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<tr>
<td>BSE sheep (ic)</td>
<td></td>
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<tr>
<td>Brain</td>
<td>297 ± 3</td>
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<tr>
<td>Spleen</td>
<td>326 ± 4</td>
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<tr>
<td>BSE sheep (oral)</td>
<td></td>
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<tr>
<td>Brain</td>
<td>328 ± 4</td>
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<tr>
<td>Spleen</td>
<td>361 ± 7</td>
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<tr>
<td><strong>BSE cow</strong></td>
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<tr>
<td>Brain</td>
<td>322 ± 2</td>
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<tr>
<td>Natural Scrapie sheep (Greyface)</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>386 ± 10</td>
</tr>
<tr>
<td>Spleen</td>
<td>399 ± 10</td>
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</tbody>
</table>

* Mice observed for lifetime i.e. up to 900 days

** Pooled data from 4 transmissions (BSE 1 - 4 given as individual transmissions in Bruce et al 1994,). Pooled homogenate from these brains was used to challenge the sheep in this study
Figure 1. "Lesion profiles" in RIII mice challenged with brain (---) or spleen (---) from:

- a. ic BSE injected sheep
- b. a BSE orally challenged sheep
- c. cow BSE: pooled data from 4 transmissions as in Table 1
- d. a natural scrapie case in a Greyface sheep

Mean vacuolation scores (+ sem) are shown in nine standard areas of mouse brain (Fraser and Dickinson 1968)