Transmission of bovine spongiform encephalopathy and scrapie to mice

H. Fraser,¹ M. E. Bruce,¹ A. Chree,¹ I. McConnell¹ and G. A. H. Wells²

¹Institute for Animal Health, AFRC and MRC Neuropathogenesis Unit, West Mains Road, Edinburgh, EH9 3JF and
²Central Veterinary Laboratory, New Haw, Weybridge, Surrey, KT15 3NB, U.K.

Transmission from four cases of bovine spongiform encephalopathy (BSE) to mice resulted in neurological disease in 100% of recipient animals, after incubation periods of between 265 and 700 days post-injection. The results from the four cases were very similar to one another. There were major differences in the incubation period between the four inbred strains of mice tested, and even between strains of the same Sinc genotype, and the incubation periods of Sinc heterozygote mice were much longer than those for any of the inbred strains. Transmission from a case of natural scrapie differed in two important respects: there were no differences in the incubation period between mouse strains of the same Sinc genotype, and that of the heterozygotes was between those of the Sinc homozygotic parental strains. The distribution of vacuolar degeneration in the brains of mice infected with scrapie also differed from those infected with the BSE isolates. Transmission was also achieved from formol-fixed BSE brain. These results show that the same strain of agent caused disease in the BSE cases, and that the relationship of BSE to scrapie in sheep is unclear.

Introduction

Bovine spongiform encephalopathy (BSE) is a neurodegenerative disease of adult cattle similar to scrapie in sheep and goats. It became epidemic in the British Isles following the first recognized cases in 1985 (Wells et al., 1987) and by November 1991 over 30000 cases had been reported (Wilesmith et al., 1991; personal communication). It is experimentally transmissible to cattle, pigs, sheep and goats, and to laboratory mice (Dawson et al., 1990, 1991; J. D. Foster, personal communication; Barlow & Middleton, 1990; Fraser et al., 1988). Very similar naturally occurring encephalopathies have been described in mink (Hartsough & Burger, 1965; Marsh et al., 1991), mule deer and Rocky Mountain elk (Williams & Young, 1980, 1982) in the U.S.A., and in a nyala (Jeffrey & Wells, 1988), an oryx, a kudu (Kirkwood et al., 1990) and the domestic cat (Wyatt et al., 1991) more recently in the U.K.

The origin of BSE and of recently recognized scrapie-like diseases in other species remains uncertain. The most likely explanation is that they arose from the use of carcass waste, originally from scrapie-infected sheep but also later from BSE-infected animals, incorporated into meat and bone meal in the diet. Scrapie agents are known to undergo mutations, and the selection of mutant strains is thought to occur when scrapie is transmitted from one species to another (Bruce & Dickinson, 1979, 1987; Kimberlin et al., 1987, 1989). One possibility is that a cattle-selected mutant of sheep scrapie, generated in apparently infected cattle, was the direct cause of the present outbreak. Transmission of scrapie to mice and subsequent serial passage in mice provide the basis for identifying and distinguishing heritable strain differences in the scrapie agent (Bruce et al., 1991; Bruce & Fraser, 1991) and therefore an approach towards identifying the possible origin of similar diseases in other species (Fraser et al., 1991).

The purpose of this paper is to describe primary transmissions from four cases of BSE and one of scrapie to mice, as an essential prelude for strain-typing the infection from different species. Mice carry a gene called Sinc which exerts a major effect on the incubation period of all scrapie strains that have been tested and provides the basis for strain-typing (Dickinson et al., 1968; Bruce et al., 1991). Scrapie strains also differ in the distribution of vacuolar degeneration produced in the brain, as represented by the 'lesion profile' (Fraser & Dickinson, 1973). The present study also provides a basis for the bioassay of the infection in the tissues of infected cattle, and for studies of the epidemiology and natural history of BSE and scrapie.

Methods

Mouse strains. Four fully inbred strains were used; RIII/FaDk (henceforth referred to as RIII) and C57BL/FaBtDk (C57BL) are both Sinc s7 homozygous strains, and VM/Dk (VM) and 1M/Dk (1M) are
both Sinc p7 strains; C57BL x VM F1 crosses were also used. Males and females were used in approximately equal numbers, and group sizes ranged from 18 to 36.

Sources of scrapie/BSE infection. The BSE sources were brains of four Friesian Holstein cows affected with the clinical disease in 1987, from farms in different parts of England. This source material has also been used in transmission experiments to cattle (Dawson et al., 1990, 1991). The scrapie source was from the brain of a Greyface ewe killed in 1985 in the terminal stages of the disease, and came from southern Scotland. The clinical diagnosis of all these cases was confirmed histopathologically.

Injections. Cow or sheep brain was homogenized in physiological saline at 10% concentration. Four- to 7-week-old mice were injected intracerebrally (i.c.) with 0.02 ml of homogenate and intraperitoneally (i.p.) with 0.1 ml. For subpassage, mouse brain was prepared as a 1% homogenate which was centrifuged at 500 g prior to i.c. injection. Prolonged (2 years) formol-fixed brain material was leached in saline solution for 24 h prior to 10% homogenization and i.c. and i.p. injection.

Incubation period measurement. Injected mice were coded and scored weekly for the occurrence of neurological signs of scrapie-like illness. They were killed at a defined clinical endpoint, either when they had shown progressive clinical signs over a period of 3 consecutive weeks or when they were in extremis (Dickinson et al., 1968). After deceding, incubation periods were calculated as the interval between injection and the defined endpoint.

Titrations. Homogenate from one of the BSE sources was diluted by serial 10-fold dilutions to 10^-4 and injected into groups of 12 mice to obtain a titration endpoint, calculated according to the method of Kärber (1931). Titrations were carried out in C57BL and RIII mice, using the i.c. injection route to establish whether differences in incubation period with different strains of mice are due to intrinsic differences in infectability and also to provide a dose–response curve for subsequent indirect estimates of infectivity with single dilutions (Dickinson et al., 1969).

Histopathological procedures. Mice were killed by cervical fracture and their brains were immersion-fixed in 10% formol saline. Haematoxylin- and eosin-stained 6 µm paraffin sections were scored for the intensity of vacuolar degeneration on a scale of 0 to 5 in nine defined grey matter areas and on a scale of 0 to 3 in three white matter areas. After deceding, lesion profiles were constructed from the average score in each area (Fraser & Dickinson, 1968; Fraser, 1976). The ultrastructural neuropathology of the lesions of BSE in mice is published elsewhere (Jeffrey et al., 1992).

Results

Primary transmissions

Transmissions were achieved from all four BSE cases and from the one scrapie case, with 100% incidence in all mouse strains excluding occasional losses due to intercurrent illness. The incubation periods and neuropathology revealed that the four BSE cases were very similar but differed from the scrapie case. There were major differences in the incubation period between the four inbred strains of mice injected with BSE, and between mice of the same Sinc genotype (Table 1). The differences in mean incubation period (80 to 120 days) between the two Sinc s7 strains, and those (43 to 66 days) between the two p7 strains with BSE are highly significant (P < 0.001 based on two-tailed t-test for all comparisons of BSE). The incubation period of BSE in Sinc s7p7 heterozygotes (C57BL x VM) was very much longer than that in both the parental and other strains. The results with the scrapie case showed major differences from BSE: the incubation periods were 60 to 250 days longer than with BSE in three of the strains, with only insignificant (23 days between the s7 and 40 days between the p7 mice) differences between mice of the same Sinc genotype (P > 0.05 in both comparisons), whereas the incubation period in the Sinc heterozygote mouse was intermediate between those of the parental strains. Transmission from formol-fixed brain from BSE 3 and BSE 4 to C57BL and RIII mice resulted in about 40 to 80 day longer incubation periods, maintaining a highly significant (P < 0.001) 100 day difference between the RIII and C57BL mouse strains. In all these results there is very little variation within groups of mice, with standard errors rarely exceeding 2% of the mean incubation period, indicating close host control of the infective and replicative events leading to clinical illness.

The neuropathology in the affected mice consisted of a characteristic vacuolar degeneration in areas of grey matter in brain and spinal cord (Fig. 1a and b). Vacuolation in white matter tracts was inconspicuous. Asymmetrical vacuolation (Fig. 1c) and amyloid plaques (Fig. 1d) occurred in VM, IM (Table 2) and C57BL x VM mice (data not shown). The lesion profiles (Fig. 2) in BSE-infected mice differed between mouse strains, but within each strain there were few differences between the BSE cases. In VM and IM mice the lesion profiles of the scrapie transmission were broadly similar to the BSE transmissions, with prominent degeneration in the hindbrain, but in C57BL and RIII mice the lesion

<table>
<thead>
<tr>
<th>Source (Sinc genotype)</th>
<th>C57BL (s7s7)</th>
<th>RIH (s7s7)</th>
<th>VM (p7p7)</th>
<th>IM (p7p7)</th>
<th>C57BL x VM (s7p7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE 1</td>
<td>438 ± 7*</td>
<td>328 ± 3</td>
<td>471 ± 8</td>
<td>537 ± 7</td>
<td>743 ± 14</td>
</tr>
<tr>
<td>BSE 2</td>
<td>407 ± 4</td>
<td>327 ± 4</td>
<td>499 ± 8</td>
<td>548 ± 9</td>
<td></td>
</tr>
<tr>
<td>BSE 3</td>
<td>436 ± 6</td>
<td>316 ± 3</td>
<td>518 ± 7</td>
<td>561 ± 9</td>
<td></td>
</tr>
<tr>
<td>BSE 3 (FF)†</td>
<td>474 ± 10</td>
<td>379 ± 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSE 4</td>
<td>423 ± 5</td>
<td>314 ± 3</td>
<td>514 ± 11</td>
<td>565 ± 8</td>
<td></td>
</tr>
<tr>
<td>BSE 4 (FF)</td>
<td>507 ± 24</td>
<td>391 ± 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scrapie</td>
<td>404 ± 5</td>
<td>381 ± 11</td>
<td>769 ± 16</td>
<td>809 ± 25</td>
<td>611 ± 8</td>
</tr>
</tbody>
</table>

* Incubation period; days, mean ± s.e.m.
† FF, Formol-fixed.

Table 1. Incubation periods in four mouse strains and one F1 cross injected i.c. and i.p. with brain homogenates from four cases of BSE, including formol-fixed brain, and one from a case of scrapie.
profiles of the BSE- and scrapie-infected mice differed markedly, with severe involvement of the prosencephalon in the scrapie-infected mice. This more rostral lesion distribution was a particularly prominent feature in RIII mice, in which the degeneration in the optic tectum, hypothalamus, thalamus, hippocampus, septum and cerebral cortex was particularly severe. A more detailed account of the neuropathology will be the subject of a separate publication.

Subpassage from a BSE-infected VM mouse in the primary transmission, which had an incubation period of 433 days, gave a mean (± s.e.m.) incubation period in

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**Table 2. The incidence of asymmetrical vacuolar degeneration and of amyloid plaques in mice affected with BSE or scrapie**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>BSE 1</th>
<th>BSE 2</th>
<th>BSE 3</th>
<th>BSE 4</th>
<th>Scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P*</td>
<td>AS*</td>
<td>P</td>
<td>AS</td>
<td>P</td>
</tr>
<tr>
<td>C57BL</td>
<td>0/16†</td>
<td>0/16</td>
<td>0/20</td>
<td>0/20</td>
<td>0/15</td>
</tr>
<tr>
<td>RIII</td>
<td>0/33</td>
<td>0/33</td>
<td>0/32</td>
<td>1/32</td>
<td>0/29</td>
</tr>
</tbody>
</table>

*AS, Asymmetrical vacuolar degeneration; P, amyloid plaques.
† Number identified microscopically in five standard haematoxylin- and eosin-stained sections. Numerator, number of affected brains; denominator, number of brains examined.
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Fig. 2. Lesion profiles in grey matter from mice clinically affected with neurological disease following infection with scrapie from a Greyface sheep or with BSE from four sources. (a) C57BL mice; (b) RIII mice; (c) VM mice; (d) 1M mice. Scrapie (----); BSE 1 (-----); BSE 2 (-----); BSE 3 (-----); BSE 4 (-----). Brain areas are: 1, medulla; 2, cerebellum; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, paraterminal body; 8, posterior cerebral cortex; 9, anterior cerebral cortex. Lesion scoring is on a scale of severity of vacuolation 0 to 5, but is shown only up to a score of 4 for the results in this paper (for details see Fraser & Dickinson, 1968).

VM recipients of 116 ± 1 days. This is the shortest incubation period for any scrapie strain or isolate in normal mice.

**Titration experiment**

Titrations in C57BL and RIII mice using the i.c. injection route above are reported (Table 3). At a high dose (10⁻¹ dilution) the incubation periods following i.c. injection were 25 to 56 days longer for C57BL and 35 to 49 days longer for RIII mice than when i.c. and i.p. routes were combined (Tables 1 and 3). No differences in titre endpoints were found between the mouse strains, and a 62 to 93 day significant (P < 0.01 to 0.001) difference in average incubation period between RIII and C57BL mice was retained throughout the dilution series. This shows that the large difference in incubation period between the two mouse strains cannot be accounted for by differences in the efficiency of infection. The longest incubation periods in the terminal dilution groups were 490 days in RIII mice and 536 in C57BL. These results indicate that there are at least 10⁵⁻¹ to 10⁵⁻² i.c. ID₅₀ mouse infectious units per gram of terminally affected cattle brain (Kärber, 1931). Bioassays of spleen, lymph nodes, semen, buffy coat and muscle from BSE 2 have failed to produce disease in RIII mice in an observation period of over 800 days. A fuller
account of titration results in different mouse strains, routes of infection, and assays of peripheral tissues, will be included in later publications.

Discussion

On the basis of the incubation periods and lesion profiles in mice there is no evidence of variation in the phenotype of the infection in these four cases of BSE. This is consistent with the similar incubation periods produced in cattle, following combined intracerebral and intravenous infection of calves from the same four cases (Dawson et al., 1991).

More work is needed to establish whether the BSE outbreak is caused by a single type or strain of scrapie-like agent and whether there are isolates from sheep or other ruminants which can or cannot be distinguished from each other. This will depend on obtaining primary transmission data from other BSE cases at different stages of the epidemic, from a variety of sheep scrapie sources, and from scrapie-like diseases in other species. Preliminary unpublished results suggest that the infections in nyala, kudu and domestic cats are similar to BSE. Transmission from formol-fixed brain was effective in all the injected mice, but with a prolongation in incubation period, possibly as a result of titre loss (Brown et al., 1990). The difference in incubation period between C57BL and RIJJ mice, which appears to be characteristic of the BSE transmissions, was unaffected by formalin. The phenotype of an isolate from experimentally BSE-infected Cheviot sheep is also similar to BSE (J. D. Foster, personal communication). This indicates that the characteristics of the disease can be independent of the species in which it occurs. It is also apparent that the isolate from a single case of scrapie in Greyface sheep differs from the BSE cases. In addition to the one scrapie transmission from a Greyface sheep reported here, transmissions have been attempted in the Edinburgh laboratory from natural scrapie in the U.K. and Iceland from 25 sheep and one goat over the past 25 years. The results were variable, and led to the isolation on serial passage of seven strains of murine scrapie, but eight cases from sheep failed to produce recognizable neurological disease in mice observed throughout their full lifespan (Fraser, 1983; Fraser et al., 1989, 1991). Evidence of scrapie strain variation has also been obtained in natural cases in the U.S.A. (Carp & Callahan, 1991). The four BSE transmissions reported in this paper differ from all our previous scrapie transmissions at primary passage in which, to date, the 100 day incubation period separation between C57BL and RIJJ mice was never seen. It is unlikely that the single scrapie case from Scotland differed from cases in England, in which the four BSE cases arose, in view of the extensive and unrestricted movement of breeding and commercial sheep in the U.K. However, transmissions from scrapie cases throughout Britain are underway.

The major gene controlling incubation period in mice is the Sinc gene (Dickinson et al., 1968; Dickinson & Meikle, 1971; Bruce et al., 1991). The major differences in neuropathology with different strains of scrapie in mice are moderately influenced by the Sinc gene but also by other unidentified genes (Bruce et al., 1991). In the BSE transmissions reported here, differences in incubation period between mouse strains of the same Sinc genotype provide evidence for another gene or genes controlling primary BSE infection in mice. This may act by a direct genetic control of incubation period, analogous to the action of Sinc itself; this possibility is supported by finding no difference in the infectability of RIJJ and C57BL mice and the consistent 77 to 102 day difference in the incubation period throughout the dilution range (Table 3). An alternative possibility, namely that the allelic difference permits the selection and replication of different mutant strains of agent, is excluded as there are no differences in strains passaged from mice of the same Sinc genotype (Fraser et al., 1991).

Another difference between the scrapie and BSE transmissions is the incubation periods in Sinc heterozygote (C57BL × VM) mice; with BSE these have a 200 day longer incubation period than the longer incubation (VM) parent, whereas with scrapie the incubation period is intermediate between the parents. It is known that cloned strains of mouse-passaged scrapie vary with respect to their incubation periods in Sinc heterozygote mice relative to those in the parental strains (Fraser et al., 1991). However, in other primary transmissions from natural sheep scrapie the Sinc heterozygote mice have always had longer incubation periods than the longer incubation (always VM) parent, whereas from a single goat transmission the heterozygote was intermediate (Dickinson, 1976; Fraser, 1983; Fraser et al., 1989, unpublished results). The incubation period ranking between Sinc s7, p7 and heterozygote mice might indicate differences between scrapie sources and sources of scrapie-like infection, even at primary passage.

The neuropathology and lesion profiles in BSE-infected mice were almost identical from the four cases, providing further evidence that the same infecting strain was responsible for the disease in each. The neuropathology in the scrapie-infected Sinc s7 mice (RIII and C57BL) showed severe forebrain lesions which were absent from those injected with BSE. Asymmetrical degenerative vacuolation (Bruce & Fraser, 1982) and amyloid plaques (Bruce et al., 1976) were common in both p7 mouse strains in both the BSE and scrapie-infected mice. Focal asymmetrical lesions in scrapie are
frequently associated with the occurrence of mutant strains of scrapie and it is suggested that these are sites of mutant strain generation (Fraser, 1979; Bruce & Dickinson, 1979; Bruce et al., 1989). A mutant strain, known as 301V, with the shortest incubation period of any known mouse-passaged scrapie strain, has emerged on subpassage from BSE-infected VM mice (Fraser et al., 1991).

An important conclusion from these results is that primary transmission into mice is an orderly, host-controlled process of agent replication arising from primary transmission into mice is an orderly, host-passage from BSE-infected VM mice (Fraser mouse-passaged scrapie strain, has emerged on submutant strain generation (Fraser, 1979; Bruce & Dickin-son, 1979).


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References


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