

14

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Bovine Spongiform Encephalopathy

Epidemiology, Low Dose Exposure and Risks

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EPIDEMIOLOGY

Bovine spongiform encephalopathy (BSE) was identified in November 1986 as a new scrapie-like disease, which is associated with the presence of characteristic spongiform lesions in the brains of clinically affected cattle.^{1,2} Extracts of affected brains contain abnormal fibrils which, like the fibrils associated with scrapie, are derived from an abnormal isoform of the cellular protein, PrP.^{1,3} BSE is experimentally transmissible by injection of affected brain homogenates to cattle,⁴ mice,⁵ and marmosets.⁶ The disease has also been transmitted by feeding mice with infected brain, thus reproducing what is believed to be the route of infection of cattle.⁷

BSE occurs as a food-borne infection associated with the use of meat and bone meal as a protein supplement in concentrated feeds.⁸⁻¹¹ Meat and bone meal is one of the two primary products of rendering, which is a "cooking" process used in the disposal of dead stock and animal waste from abattoirs *etc.* The other product of rendering, tallow (animal fat), is not implicated as a vehicle of infection.^{8,9}

The BSE epidemic in the U.K. was initiated by a sudden increase in exposure that was sufficient to cause infection with a scrapie-like agent, sometime in the winter of 1981/82.⁹

This coincided with a period of rapid decline, for economic reasons, in the use of solvent extraction in the production of meat and bone meal. The effect of this change was to remove two partial disinfection steps for the scrapie/BSE agent: the prolonged exposure to organic solvents at high temperatures; and the subsequent removal of the last traces of solvent by treating meat and bone meal with superheated steam.⁹ The consequence was to allow enough infectivity to survive in meat and bone meal to cause an extended common source epidemic.

The epidemic has involved mostly cattle that were born in dairy herds where

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it is common to raise calves on concentrated feeds from a very early age.¹⁰ Modeling and case control studies suggest that a high proportion of all BSE cattle were infected as calves, during the first 6 months of life,¹¹ including many cross-bred animals that were subsequently sold into beef herds. Hence, the age-specific incidences of BSE reflect a minimum incubation period of about 2 years and a median of 4 to 6 years.^{10,12}

Sheep are the only generally recognized natural reservoir of scrapie-like agents in animals, and cattle are susceptible to scrapie when administered by a combination of oral and parenteral routes.¹³ Therefore, it is reasonable to assume that the BSE epidemic started with scrapie infection crossing the species barrier into cattle, via feed (but it is difficult to exclude the theoretical possibility of inapparent endemic BSE infection of cattle in the U.K. and elsewhere.^{14,15}

However, it is likely that the purely "scrapie-driven" phase of the epidemic was relatively short-lived. Most abattoirs and renderers produce and process material from sheep and cattle, and both species were represented in the bulk of the meat and bone meal used by the U.K. feed industry. Once infection had occurred in cattle, more and more of the animal waste being rendered would have come from cattle infected with BSE and this agent would have a selective advantage over the scrapie agent because there would no longer be a species barrier to infection.¹⁵ In time, the recycling of bovine infection would dominate the epidemic by artificially creating an increasingly large reservoir of BSE infection in the cattle population. The 17-fold increase in the observed annual incidence of BSE cases from 1988 to 1992 (TABLE 1) is almost certainly the consequence of recycling during the "BSE-driven" phase of the epidemic.¹⁰ The major effect of recycling on the epidemic probably started in 1984/85, before clinical BSE had occurred.¹⁴

The nature of the species barrier and the effect of "serial passage" in cattle that recycling would produce give rise to testable predictions that have been discussed elsewhere.¹⁵ Briefly, two main factors are involved in the interspecies transmission of the scrapie family of agents. One, the "donor species" effect,¹⁶ is related to differences in the *PrP* gene sequence of the species infected and that exposed.¹⁷ The other factor is the strain of agent which can influence the ease or difficulty of interspecies transmission to such an extent that experimental passage across the species barrier in laboratory animals provides one of the few practical ways of isolating mutant strains.^{16,18} Recycling would rapidly select whichever common

TABLE 1. Some Epidemiological Data on Bovine Spongiform Encephalopathy (BSE) in the United Kingdom

	1988	1989	1990	1991	1992 ^a
Number of cases confirmed	2,184	7,136	14,181	25,026	36,617
% Incidence in breeding animals ^b	.05	.16	.32	.57	.83
Within herd incidence ^c	1.8	2.0	2.1	2.3	2.7
	1.8	1.9	2.2	2.5	—
Number of new herds with BSE ^d	—	1,780	2,576	2,522	3,492
	1,462	2,051	2,234	3,450	—

^a Provisional figures for 1992.

^b Derived from the total number of confirmed BSE cases that occurred each year and the total population of cattle of more than 2 years of age; approximately 4.4 million.

^c For all herds with 1 or more cases of BSE. Data are shown for consecutive 6-monthly periods, January to June and July to December.

^d Data are shown for consecutive 6-monthly periods as in (c) above.

strain of the scrapie agent in sheep was most able to cross the species barrier into cattle. And the continued recycling of this agent in cattle would favor the selection of any mutant strain, derived from it, which had a quicker replication time and shorter incubation period.¹⁵

Strain-typing studies of 4 geographically separate isolates of BSE, collected at the end of 1987, suggest that the BSE epidemic involves one major strain of cattle-adapted agent.⁵ This accounts for the striking uniformity in the distribution of vacuolar lesions in BSE brain, compared to sheep scrapie.² Moreover, the BSE strain appears to be different from known scrapie strains.⁵

In July 1988, the British government banned the feeding of ruminant-derived protein to all ruminants.¹⁹ This was to prevent further scrapie and BSE infections, via feed, of cattle, sheep, goats and exotic species of ruminants kept in zoos, some of which had developed spongiform encephalopathy from the same type of feeds implicated in BSE.^{8,20-23} In practice, potentially contaminated feeds that had been manufactured before the ban were used up over a period of several months afterwards. Because of this and protracted incubation periods, the epidemic was only approaching a plateau at the time of writing (May 1993). But the last two years have seen a marked reduction of BSE cases in animals in the 2-3 and 3-4 year age groups. The expected downturn in the epidemic should occur once there is a reduction in cases in 4- to 6-year old animals, which dominated the epidemic.^{24,25}

It is premature to exclude the possibility of maternal or lateral transmission of BSE infection, as happens with sheep scrapie.²⁶ However, there is still no clear evidence that either occurs,^{24,25} and it seems increasingly likely that any natural transmission of BSE would be insufficient to sustain the epidemic in cattle.^{14,26} The continuation of the ruminant protein feed ban may be all that is necessary to eradicate BSE from the U.K.

LOW-DOSE EXPOSURE

The earliest known cases of BSE occurred sporadically throughout England and Wales.¹² Although marked regional variations emerged subsequently,⁹ the occurrence of BSE-affected herds has been generally random and with a low average incidence of cases.

A crude illustration of this is given by the cumulative data showing that in October 1990, 5.5 years after the first recorded case of BSE (April 1985⁸), 78.4% of all herds affected by BSE had only had 1 or 2 cases (TABLE 2). In the following 30 months, when the total number of cases increased by nearly 5-fold, the proportion of affected herds with a cumulative total of 1 or 2 cases was still 59.7%.

TABLE 2. Cumulative Total Number of Confirmed Cases of BSE in the United Kingdom at Two Dates, 30 Months Apart

	1 October 1990	19 April 1993
Total number of cases	18,795	91,342
Total number of affected herds	9,129	24,720
Average number of cases/herd	2.1	3.7
% Affected herds with 1 case	59.0	41.7
% Affected herds with 2 cases	19.4	18.0

A better illustration is the continuing low within-herd incidences of BSE over successive 6-monthly periods from 1988 to 1992. The 17-fold increase in the number of BSE cases per year was mainly due to an increase in the number of new herds experiencing cases of BSE, rather than an increase in the within-herd incidence (TABLE 1).

There are two types of explanation for the low incidence of BSE. The first is that clinical cases occur only in animals of an uncommon susceptible genotype, perhaps akin to the association of cases of natural scrapie with specific variants of the sheep *Sip (PrP)* gene.²⁷ Variations in the nucleotide sequence of the bovine *PrP* gene have been found,²⁸ but no specific associations with BSE have been reported. Neither has the occurrence of BSE in different breeds of dairy cattle signaled the importance of genetic factors, nor have epidemiological studies of BSE in large herds.^{8,29} Particularly impressive is the uniform susceptibility and incubation period in two quite different breeds of cattle after the injection of BSE infected brain.⁴ This observation is in such stark contrast to the different responses of sheep injected with scrapie as to exclude genetic variation in cattle as a major factor in the occurrence of BSE.¹⁵

The second and far more plausible explanation for the low incidence of BSE is that the average exposure to infection in feed has been at a very low level, analogous to bio-assays of scrapie infectivity at limiting doses when cases occur infrequently and unpredictably. Low-dose exposure is consistent with the main effect produced by the recycling of infection in cattle which was to increase the amount of meat and bone meal with a minimum "threshold" dose of agent to cause disease,¹² with only a modest increase in the average concentration of infectivity in contaminated batches of feed, as reflected by the within-herd incidences of BSE (TABLE 1).

Some idea of the amount of contamination of calf rations can be obtained by considering the model used to estimate the attack rate in an affected herd.¹² If an average dairy farm has 70 adult cows and the annual replacement rate is 20%, then each birth cohort would contribute 14 heifers to the adult herd. Assuming calfhood exposure, 7 cases in a cohort (that is, 50%; a comparatively rare occurrence) would be the equivalent of an average effective exposure of 1 oral LD₅₀ per calf of cattle-adapted BSE agent (that is, with no species barrier). During the first 3 months of life, a calf might be fed 70–75 kg of concentrated feeds and about a tonne of feed would be needed to rear 14 calves. Therefore, it is conceivable that the average contamination of an infected batch of feed might have been as low as 14 oral LD₅₀ per tonne.

It must be stressed that this estimate rests on several assumptions. One important assumption is that infectivity was uniformly distributed within batches of contaminated feed. This is most unlikely. Contamination would probably have been in the form of infected "packets,"¹² in some of which the amount of infectivity could have been high. Given the apparent absence of genetic factors influencing BSE, the incidence of cases in exposed birth cohorts would have depended on the concentration of infected packets in different batches of feed. However, large variations in the amount of infectivity within individual packets should be reflected as differences in incubation period (if these can be estimated with sufficient precision), and useful comparisons might be possible with dose-incubation data from cattle experimentally infected with BSE, orally. These analyses are being attempted.

The concept of low-dose exposure provides the key to understanding several aspects of the BSE epidemic. For example, the cumulative total number of BSE cases is currently (May 1993) just under 100,000, and the peak annual incidence

will be close to 1% (TABLE 1). The reasons why the epidemic became so large are because rations containing sufficient amounts of BSE agent to cause disease were fed to a substantial and increasing proportion of the U.K. cattle population, until the ruminant protein feed ban became effective in 1988/89, and because cattle appear to be uniformly susceptible to BSE.

However, at the beginning of the epidemic, exposure was presumably to scrapie and across a sheep-cattle species barrier. Depending on the extent of this barrier, the effective exposure of cattle to scrapie could have been less than to cattle-adapted BSE, and the incubation period longer.²⁶ Given that the average age of dairy cattle in Britain is just over 5 years, a combination of very low effective exposure and prolonged incubation period may have made the "scrapie-driven" phase of the BSE epidemic almost invisible: perhaps less than the annual incidence of "retrospective cases" of 1 in 270,000 adults, which occurred between April 1985 and July 1986.⁸

These considerations are relevant to the possible occurrence of BSE in other countries which have endemic scrapie and broadly similar rendering and feed practices to those in the U.K. The effectiveness of surveillance for clinical disease will be influenced greatly by the age of potential exposure (calves or adults) and the age structure of the sentinel population (dairy cows). And the initiation of a BSE epidemic from scrapie will be critically dependent on the exposure being high enough.

RISKS OF BSE IN OTHER COUNTRIES

In retrospect, it seems likely that the start of the BSE epidemic in the U.K. was a consequence of the *simultaneous* presence of four main factors: (1) a large sheep population, relative to that of cattle, with both populations widely distributed; (2) a high incidence of endemic scrapie infection; (3) the use of substantial quantities of ovine meat and bone meal in cattle feed; (4) conditions of rendering that allowed the survival of small but significant amounts of infectivity, which would have depended on the amount of the initial contamination.^{12,26}

Quantitative analysis of these factors (particularly 1 and 3) has been carried out in several countries, two of which have published formal risk assessments: the U.S.A.³⁰ and Argentina.³¹ The evidence is that no other country possesses the same combination of risk factors due to scrapie as occurred in the U.K., even those which have reported cases of BSE.¹²

The first case of BSE to be identified outside mainland Britain occurred in Northern Ireland, in July 1988, about 3 years after the first known case in Britain (April 1985). Studies suggest that BSE did not arise in Northern Ireland from indigenous scrapie,³² but was most probably due to a "spillover" of infection, from Britain, in the form of exports of live animals and feedstuffs infected with the BSE agent, and to the recycling of infection until the use of ruminant protein was banned there in 1989. To date (May 1993), 809 cases have been reported.

This "spillover" may have contributed to the occurrence of BSE in the Republic of Ireland where the first case was reported a year later (1989). But there is insufficient information on the possible origin of all 71 cases, which have now been confirmed, to conclude that an indigenous source of infection was not also present.

The later occurrence of BSE cases in France (5 since 1991) is possibly due to imports of infected feedstuffs from the U.K., even though no clear link has been

established. A link with the 34 BSE cases that have been confirmed in Switzerland since 1990 is even more tenuous because very little animal protein has been imported from the U.K. However, the Swiss imported considerable quantities from France and elsewhere, and the international trade in animal protein is known to have continued until the end of 1989.

The only other countries which have reported cases of BSE are the Sultanate of Oman,² the Falkland Islands,¹ and Denmark.¹ All of these cases occurred in cattle imported from the U.K., before the feed ban was introduced.^{26,33}

In conclusion, there is no evidence that the occurrence of multiple cases of BSE in continental Europe marks the beginnings of major epidemics arising from endemic scrapie. Some of the cases that have occurred outside Great Britain resulted from the movement of British cattle which were already infected. Others could have arisen from the consumption of ruminant protein imported from Britain, although no direct link has been established. However, it is impossible to exclude, totally, a risk from endemic scrapie in other countries because unfavorable combinations of the scrapie-related risk factors might occur locally. But with the passage of time, these risks seem increasingly low.

RISKS TO OTHER SPECIES

Relatively few tissues support the replication of the scrapie agent to high titers, even at the clinical stage of natural scrapie in Suffolk sheep and goats (TABLE 3). At the start of the BSE epidemic, the main sources of scrapie agent in meat and bone meal would have been brain (sheep heads) and intestines (green offals; which include the ileum, proximal colon and other regions that contain large amounts of Peyer's patches in which the scrapie agent multiplies.^{35,36} A similar group of bovine tissues was expected to be the main potential source of infection during the "BSE-driven" phase of the epidemic.

In 1988, the introduction of the ruminant protein ban was a simple, practical measure to protect all ruminant species from food-borne infections with either scrapie or BSE, regardless of the tissues of greatest risk. An assessment of the potential risks of BSE to man was based partly on the data shown in TABLE 3, but particularly on a substantial body of epidemiological evidence, collected world-wide, showing that the occurrence of human Creutzfeldt-Jakob disease (to which scrapie and BSE are related) is not associated with scrapie, sheep or the consumption of sheep products.^{37,38}

This is an important observation because, historically, many people in many countries have eaten tissues from scrapie-infected sheep, including brain.³⁹ Some idea of past exposure to scrapie can be obtained by considering the consumption of a lightly cooked sheep brain from an incipient scrapie case. If the titer of agent in sheep brain (see TABLE 3) is rounded up to 10 million i.c. LD₅₀ units/g to allow for an underestimation of titer measured across a sheep-mouse species barrier, the consumption of a whole sheep brain (about 100 g) would constitute a dose of around 1 billion i.c. LD₅₀ units. If it is then assumed that the oral/alimentary route of exposure of man is as inefficient as it is with the 139A strain of mouse scrapie in mice (about 100,000 times lower than infection by the i.c. route: TABLE 4), the effective exposure of man would still be about 10,000 oral LD₅₀ units. However, a smaller difference between the i.c. and oral routes, say 1,000-fold, would increase this effective exposure to about 1 million infectious units.

These calculations illustrate the likely extent of the sheep-man species barrier

TABLE 3. Infectivity Titers (Bio-assayed in Mice) in Tissues from up to 9 Suffolk Sheep (34-57 Months Old) and up to 3 Goats (38-49 Months Old), Naturally Affected with Scrapie

Tissues	Titers (mean \pm SEM of (n) samples) ^a	
	Scrapie Sheep	Scrapie Goats
<i>Highest infectivity</i>		
Brain	5.6 \pm 0.2 (51)	6.5 \pm 0.2 (18)
Spinal Cord	5.4 \pm 0.3 (9)	6.1 \pm 0.2 (6)
<i>High infectivity</i>		
Ileum	4.7 \pm 0.1 (9)	4.6 \pm 0.3 (3)
Lymph nodes	4.2 \pm 0.1 (45)	4.8 \pm 0.1 (18)
Proximal Colon	4.5 \pm 0.2 (9)	4.7 \pm 0.2 (3)
Spleen	4.5 \pm 0.3 (9)	4.5 \pm 0.1 (3)
Tonsil	4.2 \pm 0.4 (9)	5.1 \pm 0.1 (3)
<i>Moderate infectivity</i>		
Adrenal	<2.8 \pm 0.3 (9)	4.3 \pm 0.2 (3)
Distal Colon	<2.7 \pm 0.2 (9)	3.3 \pm 0.5 (3)
Nasal mucosa	<2.3 \pm 0.2 (9)	3.6 \pm 0.2 (3)
Pituitary	<2.5 \pm 0.2 (9)	4.9 \pm 0.1 (3)
Sciatic nerve	3.1 \pm 0.3 (9)	3.6 \pm 0.3 (3)
<i>Minimal infectivity</i>		
Bone marrow	<2.0 \pm 0.1 (9)	<2.0 (3)
Cerebrospinal fluid	<2.2 \pm 0.2 (9)	<1.9 \pm 0.4 (3)
Liver	<2.0 \pm 0.1 (9)	—
Lung	<2.0 (9)	<2.1 \pm 0.1 (2)
Pancreas	<2.1 \pm 0.1 (9)	—
Thymus	<2.2 \pm 0.2 (9)	<2.3 \pm 0.2 (3)
<i>No detectable infectivity</i>		
Blood clot	<1.0 (9)	<1.0 (3)
Faeces	—	<2.0 (3)
Heart muscle	<2.0 (9)	—
Kidney	<2.0 (9)	<2.0 (3)
Mammary gland	<2.0 (7)	<2.0 (3)
Milk	—	<1.0 (3)
Ovary	<2.0 (7)	<2.0 (3)
Saliva	<1.0 (9)	—
Salivary glands	<2.0 (9)	<2.0 (3)
Seminal vesicle	<2.0 (1)	—
Serum	—	<1.0 (3)
Skeletal muscle	<2.0 (9)	<1.0 (1)
Testis	<2.0 (1)	—
Thyroid	<2.0 (9)	—
Uterus	<2.0 (3)	<2.0 (3)

^a Titers are expressed as arithmetic means of log 10 mouse *i.e.* LD₅₀/g or ml of tissue: re-analysis of data from Hadlow and others.^{34,35}

to scrapie. A quantitatively similar barrier would be expected between cattle and man, but it is unlikely to be identical for two reasons. First, the donor species effect from cattle may not be the same as from sheep. Secondly, the transmission of infection to cattle, followed by recycling, may have selected a bovine-adapted strain of BSE that was different from naturally occurring strains of sheep scrapie (for which there is now some evidence: see EPIDEMIOLOGY).

The difficulty is the absence of a practical means of comparing the relative

TABLE 4. Comparisons of the Relative Efficiencies of Different Routes of Infection as Measured by Titration of Standard Pools of Scrapie-affected Mouse or Hamster Brain in Mice or Hamsters, Respectively

Route of Infection	Number of Titrations	Estimated Titer ^a (log)	Number of i.c. Units to Give 1 Unit by Each Route
<i>139A Scrapie in CW Mice^b</i>			
Intracerebral	14	7.03 ± .13	1
Intravenous	16	6.06 ± .11	9
Intraperitoneal	50	4.40 ± .06	430
Subcutaneous	11	2.64 ± .11	24,500
Intragastric	7	2.03 ± .19	100,000
<i>263K in Golden Hamsters^c</i>			
Intracerebral	26	8.50 ± .10	1
Intraperitoneal	2	3.90 ± .10	40,000

^a Titers are expressed as arithmetic means ± S.E.M of log 10 LD₅₀/30 mg of mouse brain or 50 mg of hamster brain

^b Data from Kimberlin & Walker.^{36,40}

^c Data from Kimberlin & Walker.^{41,42}

"non-transmissibilities" of scrapie and BSE to man. The uncertainty was addressed by excluding from human food the potentially high risk bovine offals, based on the data in TABLE 3. In practice the proscribed offals were limited to brain, spinal cord, intestines, spleen, thymus, and tonsil.⁴³ Thymus was included in these "specified bovine offals" because of its widespread use which counts towards the meat content of uncooked processed foods. The ban was applied to all cattle over 6 months of age at slaughter, regardless of their potential BSE status. A year later, when the first cases of spongiform encephalopathy in the domestic cat were reported^{23,44,45} the ban on the use of the specified bovine offals was extended to include all species of mammal and bird.⁴⁶

Important findings have been made since the specified bovine offals ban came into effect. The view that spongiform encephalopathy in domestic cats was due to BSE, not scrapie,²⁶ is supported by evidence associating one case of the feline disease with the same strain of BSE agent that has affected cattle.⁵ Not surprisingly, a similar strain of agent is also implicated in a case of spongiform encephalopathy in a nyala and one in a kudu.⁵

Brain stem tissue from a clinical case of BSE has been found to contain a titer of 5.2 log 10 i.c. LD₅₀/g,⁵ which is similar to the titers in scrapie sheep brain (TABLE 3: however, the possible underestimation of titer across the species barrier would not necessarily be the same for sheep scrapie as for BSE). To date, BSE infectivity has not been detected in bovine spleen or lymph nodes, suggesting that, compared to scrapie, BSE infectivity in the extra-neural tissues is proportionately much lower than is found in brain. And, as expected, no infectivity has been detected in muscle tissues from a BSE case.⁵

SUMMARY

BSE has occurred in the U.K. as an extended common source epidemic since 1985/86. The vehicle of infection was concentrated feeds containing meat and bone

meal produced by the rendering of ovine, bovine and other animal wastes. The epidemic was probably initiated in 1981/82 when a sudden decline in the use of solvents in rendering allowed a low incidence of scrapie-like infection to occur in cattle. However, the presence in feed of bovine material that, from 1984/85 (or earlier), was increasingly infected with a cattle-adapted strain of agent amplified the epidemic greatly. Nevertheless, the incidence of BSE cases nationally has been low because of the generally low effective exposure of cattle to infection in feed. This, and a combination of risk factors that were probably unique to the U.K. can explain why relatively few cases of BSE have occurred in other countries. The feeding of ruminant-derived protein to all species of ruminants was banned in Great Britain in 1988, and in Northern Ireland in 1989. A more selective approach was subsequently adopted to minimize the risks of BSE infection of other species, including man. This was based on excluding from food a small number of bovine offals whose use and predicted infectivity titers would constitute the greatest potential source of infection. Recent studies of BSE support the basis of the specified bovine offals ban and suggest that more tissues were restricted than may have been necessary.

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