

# MINIREVIEW

## Lingering Doubts about Spongiform Encephalopathy and Creutzfeldt-Jakob Disease

HARASH K. NARANG<sup>1</sup>

*Ken Bell International, Newcastle Upon Tyne NE2 3DH, United Kingdom*

Bovine spongiform encephalopathy (BSE) is an infectious disease and has been transmitted orally to many other animals, including humans. There is clear evidence of maternal transmission, although disagreement on the source of the BSE agent remains. The current theories link the origin of BSE to common scrapie in sheep. Twenty different strains of the scrapie agent have been isolated from sheep. A search of the literature indicates two distinct clinical syndromes in sheep, both of which have been called scrapie. I have designated these Type I (the common type), which exhibits itchininess and lose their wool, and Type II, which exhibits trembling and ataxia. Sheep inoculated with BSE develop Type II scrapie and they exhibit trembling. When cattle or mink are injected with the Type I strain, only a few will develop a clinical disease. By contrast, no clinical disease has so far been shown in cattle or mink by feeding them with Type I-infected sheep brains. However, either by injecting or feeding with the BSE strain, 100% of calves and mink develop the clinical disease. Evidence suggests that Type II is the cause of BSE. Identical clinical signs of Type II trembling are found in kuru and many of the recent cases of Creutzfeldt-Jakob disease. The BSE agent has caused spongiform encephalopathies (SEs) in domestic cats, tigers, and in some species of ruminants in zoos. The nature of the BSE agent remains unchanged when passaged through a range of species, irrespective of their genetic make up, demonstrating that variations in the host PrP gene are not a major factor in the susceptibility to the BSE agent. Since more than 85 zoo animals of many species have been diagnosed with SEs, from these studies it seems reasonable to conclude that the BSE agent can infect almost all mammalian species, including humans. For eradication of BSE and to reduce the risk of infection to humans, the development of a vaccine against BSE is suggested. Such a possibility should be fully explored.

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**Key words:** bovine spongiform encephalopathy; Creutzfeldt-Jakob disease; nemavirus; protease-resistant protein (PrP); scrapie; scrapie-associated fibril; spongiform encephalopathy

The classic example of spongiform encephalopathy (SE) is scrapie, a chronic wasting disease of the central nervous system (CNS) occurring in sheep, while bovine spongiform encephalopathy (BSE) in cattle is the most recent addition to the group (1, 2). Prior to 1985, five other naturally occurring scrapie-like subacute diseases were recognized. These are Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Strussler-Scheinker syndrome (GSSS), transmissible mink encephalopathy (TME), and wasting disease of mule deer and elk (1-3). They are always progressive and fatal. These experiments and the natural spread of BSE and the diversity of host species have demonstrated beyond doubt the infectious transmissible nature of the disease. All SEs can be experimentally transmitted from one species to another and, therefore, the term transmissible spongiform encephalopathies (TSEs) is a valid term. Until the emergence of BSE, the TSEs were considered of little or no importance by medical or veterinary clinicians.

Shortly after the discovery of BSE in cattle, it was realized that the BSE agent had broken down "species barriers" and had infected many other animal species, including domestic cats (4), and captive wild animals in the British Isles (5, 6) via contaminated food. So far, 85 zoo animals of many species have been diagnosed with TSEs, with France having the highest number. The most common species of zoo animals include nyala, eland, greater kudu, members of subfamily hippotraginae, Arabian oryx, and members of the family felidae: three cheetah, puma, and ostriches (7).

In 1996, a Kent farmer in the south of England witnessed BSE on his farm and noticed that one of his 30-month-old domestic hens showed similar clinical signs as seen in BSE cattle. The first signs were behavior changes,

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<sup>1</sup> To whom requests for reprints should be addressed at Ken Bell International, 22-40 Brentwood Avenue, Newcastle Upon Tyne NE2 3DH, UK. E-mail: Harash@compuserve.com

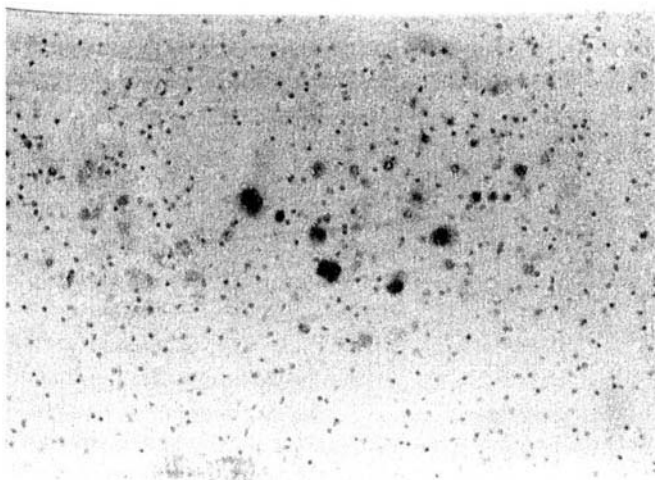
where the hen was having difficulty entering the roosting shed. As the clinical signs progressed, imbalance, uncoordination, and ataxia became well marked (2). Furthermore, two other farmers on separate farms, one in the south and one in Northumberland, reported similar cases in hens. Histopathological examination of the brains of these hens revealed reactive astrocytes with minimal vacuolation. However, immunostaining of the brain sections with PrP antibody revealed typical PrP<sup>sc</sup>-positive plaques (Figs. 1 and 2).

### The Clinical Symptoms of BSE

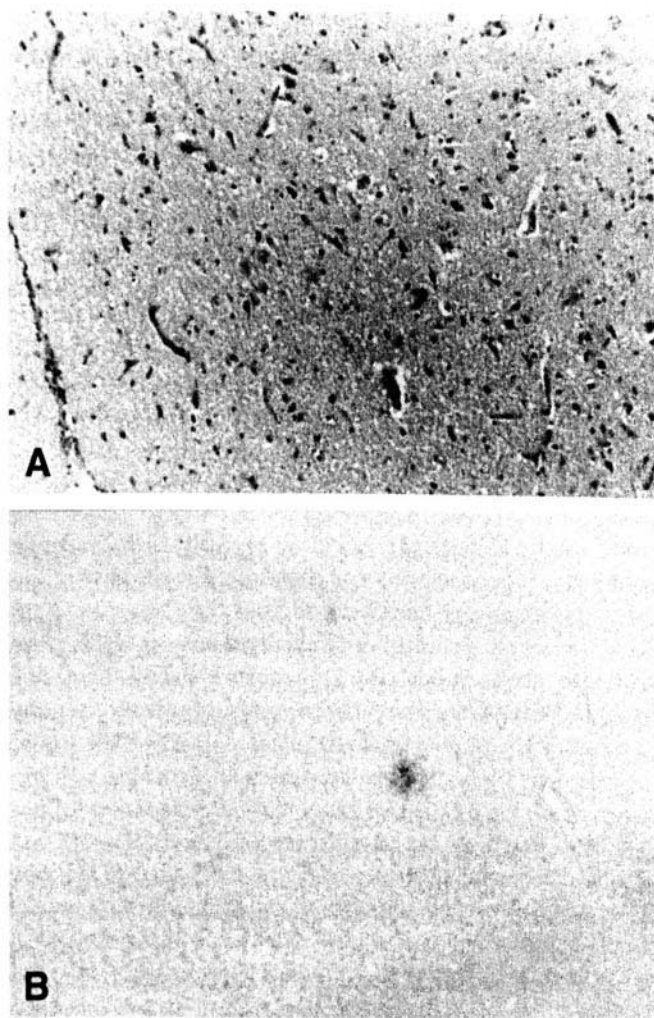
The case histories show that BSE was diagnosed in cattle previously considered in good health and with a high milk yield (8, 9). The gradual progression of symptoms has previously been described in great detail (2). The earliest symptom a farmer may observe is little more than apparently the animal being bad tempered and lost in their surroundings (2). Affected cows gradually recede from their "pecking-order" position, marked by a "loss of seniority" during the feeding and milking routine. As the clinical signs progress, the condition of the animal deteriorates and they progressively are less able to coordinate their balance. They become unmanageable, dying within 2 weeks to 6 months from the onset of symptoms. The major reported difference between cases in Switzerland and those in England has been the clinical signs at onset of the disease. A higher relative frequency of altered mental status as well as reduced milk yield and loss of weight was noted in Swiss cows (10).

### Histopathological Studies

Scrapie is diagnosed when spongy changes are found in sheep brain sections examined by microscopy. Typically, vacuoles occur in neurons of the CNS, while apparently no pathological changes are seen in other organs, even though they carry the infective agent. The extent of these vacuoles



**Figure 1.** Immunocytochemistry for PrP of a section of cerebellum from 59-year-old CJD case shows strong-staining, PrP-positive plaques. The PrP-positive plaques were seen extensively distributed throughout the cerebrum and cerebellum, a feature of the BSE agent.  $\times 250$ .



**Figure 2.** Hen suffering from spongiform encephalopathy. (A) Section of the cerebellar cortex from a case of hen showing mild vacuolation and shrunken neurons.  $\times 126$  (B) Section of the cerebellar cortex from a case of hen showing PrP immunostained-positive plaques.  $\times 250$ .

in the CNS is variable. In natural scrapie, the vacuoles are less numerous compared with an artificially induced infection (11–16). These vacuoles, "spongiform" in appearance, have become the most well-recognized diagnostic hallmarks of scrapie, BSE, and CJD.

Since 1987, several neuropathological studies on clinically suspected cases have been undertaken in England (9, 17–21). The diagnosis for BSE has been principally based on light microscopic examination of brain sections. Pathological changes relevant to diagnosis include vacuolation and prominent astrocytes hypertrophy (2). The degenerative changes are more striking in the basal ganglia and extend beyond the cerebellum (2).

### Identifying Preclinical Cases of BSE

BSE, like other TSEs, has a very long asymptomatic incubation period and a protracted clinical course of months or even years. Except for physical visual examination, the

Ministry of Agriculture Fisheries and Food (MAFF) has no diagnostic method to detect infected asymptomatic animals. In one experiment, in order to establish correlation of pathological changes with clinical signs, inoculated cattle were killed at a regular interval of 3 months. Histopathological examination of their brains revealed spongiform changes only after clinical signs appeared. Narang *et al.* (22–25) developed a “touch technique” that could reveal the scrapie-associated fibrils (SAF)/nemavirus prior to any macro symptom expression. In a random study, cow brains from apparently healthy cattle over 4 years old were collected from a local abattoir. Examination by the “touch technique” revealed that 29% of the animals were positive for SAF/nemavirus (1, 2).

At the peak of the BSE epidemic, histopathological studies of clinically diagnosed BSE cases revealed that some 7% to 13% of cattle had no typically BSE vacuolar pathology. These cases were therefore classified as clinically misdiagnosed and were termed the “negative rate” (26). Histological studies of clinically diagnosed BSE cases born after the feed ban (BABs) revealed that an increasing number (39%–57%) of brains have no significant vacuolation; however, immunohistochemical staining of the brains with the PrP antibody revealed evidence of the disease (26). In Scotland, the number of cases with no significant lesions in BABs’ gradually increased from 26% in 1988 to 1989 to 41% in December 1991 to 82% in 1993 (26). This new pattern with absence or rarity of vacuolation may reflect either the emergence of a new strain of BSE or the possibility that the infection may have occurred by a different route and has been discussed (1, 2). This feature has been observed in experimental transmission of scrapie to cattle in which inoculated calves developed clinical signs, whilst none of the brains showed typical spongiform changes seen in BSE (27).

### Detection of Protease-Resistant Protein (PrP<sup>Sc</sup> or PrP)

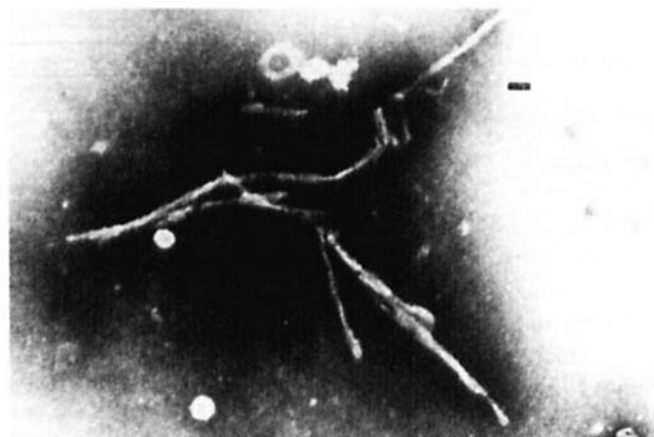
PrP<sup>Sc</sup> has been known to accumulate and form plaques in some animal species when infected with certain strains of the agent. The immunostaining for PrP with a specific PrP antibody has become another additional technique for diagnosing the disease. The distribution of PrP plaques has been applied to distinguish different strains of the TSE agent (2, 28, 29).

Immunohistochemically, the plaques have been characterized into two types. The first is amyloid protein plaques, also termed amyloid plaques, which are a “hallmark” of Alzheimer’s disease (AD). The gene coding for the precursor amyloid protein (APP) is highly conserved in evolution (30). It is transcriptionally active and is expressed in a variety of tissues and cell types (31). AD has many clinical pathological features similar to those seen in CJD. There are some studies for and against that suggest the infectious nature of the disease, as discussed in detail (32). Smith *et al.* (33) in a retrospective neuropathological study of brains

from 66 patients with AD demonstrated vacuolar changes in 50 cases (76%), which they described as almost indistinguishable histopathologically from spongiform changes characteristic of CJD.

APP plaques are also seen in patients with Down’s syndrome and “normal” aging in some humans. They also occur in about 10% cases of CJD, kuru, and Parkinson’s disease, and also in mice experimentally infected with specific strains of scrapie agent (34, 35). Amyloid plaques have been observed in 55% of sheep with natural scrapie (36), in 62% of mule deer with scrapie (37), and in only about 5% cases of BSE (19). The second plaque is protease-resistant protein (PrP, 27–30 kDa; PrP<sup>Sc</sup> or PrP) plaques that are often seen in brain sections of CJD, kuru, natural scrapie of sheep, and with some strains of experimentally induced scrapie in mice. Different strains of the TSE agent have a great influence on the number and distribution of PrP plaques. PrP 27 to 30 kDa is derived from a PrP 33- to 35-kDa precursor protein (PrP<sup>C</sup>) coded by a normal gene assigned in mice to chromosome 2 and in humans to the short-arm of chromosome 20 (38–40). It has been demonstrated that PrP molecules can aggregate to form “prion rods”/ SAF (41, 42) and they have a distinctive morphological structure in all TSEs (Fig. 3). PrP plaques have never been observed in AD or Down’s syndrome. The analysis of several different BSE brain sections revealed PrP plaques, but the sensitivity of detection appeared to be low (46.5%) (43).

While interpreting histopathological findings, consideration must be given to the passage level of the BSE agent in cattle itself and also to infection through other species such as sheep, pig, and poultry. In one study, the first passage from cattle to mice demonstrated positive transmission of the BSE agent when all infected mice developed typical clinical signs of the disease, but 55% of mice had no detectable PrP<sup>Sc</sup> or vacuoles (44). However, in subsequent passage from the mice having no detectable PrP<sup>Sc</sup> or vacuoles to having developed the clinical disease, their brain sections revealed PrP<sup>Sc</sup> and vacuoles in 100% of mice (44). Therefore, it is obvious, where typical clinical signs of the



**Figure 3.** Electron micrograph of negatively stained cerebral cortex from a clinically ill brain of a scrapie hamster prepared with grids soaked in 1% solution of SDS. Note typical SAF. Grid bar = 100 nm.

disease developed and no detectable PrP<sup>sc</sup> or vacuoles such as in cattle, other farm animals, and humans apart from intensive histopathological examination, that there will be a need for transmission studies to determine the precise causes relating to the disease.

When BSE was discovered, Will *et al.* (45) reviewed 175 sporadic CJD cases from England and 14 additional cases, aged less than 30 years old, from outside England. The authors stressed that they did not find PrP plaques nor did they see them in those young patients dying of conditions similar to CJD cases prior to the BSE epidemic. The distribution of PrP plaques in the brain has become one of the main distinguishing features in patients infected with the BSE strain of the agent (2, 29, 45). However, Narang (29, 32) reviewed 28 cases of CJD diagnosed between 1989 and 1996 in England and found that many patients had PrP plaques with similar distribution as seen in young patients, demonstrating that older patients were also infected with the BSE strain of the agent (29, 32).

### **BSE Epidemic**

Since its recognition in November 1986, BSE was believed to be caused by feeding meat and bone meal (MBM) prepared from scrapie-affected sheep. Some retrospective epidemiological studies “substantiated” the original prevalent hypothesis of food-borne infection (46–49), while no such disease existed in England before 1984. At the start of the epidemic, a marked geographical variation was observed in the incidence of BSE between northern with southern regions of England. Relatively fewer cases were reported from Scotland. Later, the number of cases in Scotland gradually increased. It was estimated that the epidemic would subside in 1992 with 20,000 cattle developing the clinical disease.

In 1985 there were only six cases of BSE in cattle, and by June 1999, there were nearly 175,000 confirmed cases of BSE cattle in England (MAFF, personal communication). It is not possible to ascertain the number of subclinical cases slaughtered between 1985 and 2000 and what proportion of such cases entered the human food chain. In 1988 the disease was made notifiable in European Union countries. Analysis of the risk factors has consistently been based upon a variety of suppositions.

At the start of the BSE epidemic, the incubation period was between 5 and 6 years and became shorter than usual when a greater proportion of 2- to 3-year-old cattle exhibited the clinical disease. The scale of the BSE epidemic in the next 2 years from 1988 to 1990 increased considerably. The total number of cases reported in June 1988 was 687; in December it was 2,160; in June 1989 it was 5,375, and in November 1989 it reached 8,100 (50). BSE cases reported in 1994 were between 400 to 500 per week. During 1996, however, a drop was observed of about 200 to 300 per week. In 1999, the average number of suspected cases reported was still around 70 cases/week. The peak monthly incidence of BSE in England in 1993 represented about 1%

of adult cattle. Though perhaps coincidental, this is similar to the maximum incidence seen in humans in the epidemic of the TSE disease, kuru, in the Fore Tribe in the 1950s (3). Only, at this stage, it was realized that the reduction in the incubation period was due to incorporation and recycling of material from animals with clinical and subclinical cases of BSE to produce MBM (46–49, 51). The major part of the BSE epidemic was, therefore, due to the artificial recycling of bovine-adapted or -selected BSE agent from scrapie-infected sheep (51).

It also became apparent that the recycling of infected feed was responsible for the selection and concentration of a unique pathogenic strain of BSE agent (1, 2, 51–53). The serial passage of the agent from cow to cow has provided an ideal equivalent situation to that observed in experimental animals. As anticipated, these results showed a gradual reduction in the incubation period after each passage. This was shown by increase of infective titre levels where the clinical disease became apparent earlier in the life span of an average cow.

On some farms in England before BSE was recognized, sheep were also being fed with small quantities of MBM. However, following the introduction of MBM ban in 1988 July for cattle, the feed became very cheap and, therefore, some farmers fed their sheep extensively with BSE-contaminated MBM. This practice produced sheep infected with the BSE agent, including those sheep breeds that were resistant to the scrapie agent. The BSE agent has, therefore, changed from being a minor strain to a major strain.

The ban on feeding MBM to cattle began in July 1988. In theory, this should have stopped infection in calves born after the ban (BABs) in 1988. However, through June 1999, 39,384 BSE cases were confirmed in cattle born after the feed ban (MAFF, personal communication). At the start of the epidemic, the number of BSE cases varied according to season, with the winter being highest. However, in BABs, the peak occurs during the summer months.

For a number of years, MAFF interpreted the occurrence of animals affected after the MBM ban as a result of MBM still being fed to cattle and classified the early BAB cases of BSE to this “Bin end” theory (54). MAFF continued to classify all cattle BAB with BSE as “horizontally” acquired infection. The accumulating evidence now available suggests that BSE cases detected after the feed ban are not due to eating contaminated food; there must have been a significant level of an additional mode of infection. It is more likely that most of BAB cases might be due to “vertical” transmission.

### **Origin of BSE**

For many, the origin of BSE remains a puzzle. Scrapie in sheep has been known for at least 250 years or more (1, 2). How had BSE suddenly and simultaneously erupted involving a very large number of cattle on many farms all over England? Why had this disease not been observed in many other countries? Is BSE due to a new “agent” or

“mutation” of the old agent? What is the true nature of the infective agent? Finally, we have to question seriously whether infected animal feed was the only source of infection.

### **Possible Source of the BSE Agent**

Traditionally in abattoirs, meat unfit for human consumption was “rendered” for animal feed and some by-products were used in other industrial processes. The rendering materials in England contained about 15% of ovine compared with about 45% of bovine origin, a ratio of 1:3 (2, 48, 49). Epidemiological studies and analysis of the situation in England revealed that the disease had rapidly spread as a result of consuming MBM contaminated with the scrapie-like agent. However, “this feed-born” hypothesis has never been experimentally tested. It is difficult to understand why these experiments have never been done.

Various hypotheses have been postulated and discussed in detail before (1, 2). The two major hypotheses discussed in this review are: bovine origin—that an unrecognized reservoir of infection existed before the outbreak of BSE in the UK cattle, and ovine origin—that the agent jumped species from scrapie-infected sheep to cattle.

It is a well-established fact that the strain of the TSE agent “breeds true” with their own particular biological properties such as incubation period and distribution of lesions. Before we consider each hypothesis as to the origin of BSE, it is important to understand the existence of different strains and their properties, as well as the main differences between them.

Since 1961, based on clinical observations, two strains of scrapie are readily recognizable in sheep: Type I, the “itchy” type and Type II, the “trotting” type (2, 55, 56). Throughout the world, Type I itchy scrapie is the most common. In Type I, majority of clinical cases are seen in the 4- to 5-year-old age group and by that time they will have had progeny of six to eight lambs in four breeding seasons. The majority of lambs born of affected sheep will develop the clinical disease (1, 2). Calves inoculated with Type I “itchy” strain develop mild spongiform changes in their brain, but the clinical signs are not similar to those observed in BSE.

Type II “trotting” strain of scrapie is very rare in sheep occurring in no more than 1 in 100,000 sheep. The sheep tremble while standing and have a peculiar gait of the forelegs known by farmers as the “cuddie trot” or “staggers.” With Type II, the clinical disease appears in the 2-year-old age group, and by that time they will only have one breeding season producing one or two lambs in which to pass down the disease. Therefore, Type II “trotting” scrapie is self-limiting. Sheep experimentally inoculated or fed with BSE brains develop Type II “trotting” scrapie and exhibit clinical signs very similar to BSE, in particular locomotor incoordination, trembling, lethargy, and ataxia.

The most important differences observed between the BSE strain and scrapie sheep are listed below. Primary passage of BSE to mice was achieved with a 100% efficiency

both by intracerebral inoculation (i.c.) and by feeding infected tissues compared with less than 50% transmission in sheep scrapie (1, 2, 57). When mice are inoculated with Type I scrapie, the incubation period for primary transmission often extends from 600 days to lifespan, while mice inoculated from cases of BSE consistently have shorter incubation periods, usually extending to about 328 days (1, 2). Scrapie sheep strains tested in sheep, goats, and mice revealed that with an intraperitoneal route of infection, the incubation periods are consistently longer than with i.c. route. Compared with the BSE strain, the route of infection has little effect, if any, on the incubation periods. Most important of all, mink, cats, and cattle never developed a clinical disease after being fed with the scrapie strains of the agent compared with 100% developing the clinical disease after being fed with the BSE agent.

### **Influence of the Agent Strain Versus Host Genetic Factors**

Some researchers have suggested that a gene termed Sinc gene in mice and Sip gene in sheep exert a major influence on the incubation period and also influence the lesion profile in brains of experimental scrapie (34, 58–60). Later, the Sinc gene and Sip gene were described as Prn-i (61, 62). In comparative transmission studies, the pattern of incubation periods and pathological lesions found were remarkably similar in BSE, cats, greater kudu, and nyala in the same Sinc genotypes mice, but different for the common scrapie (1, 2). Further, the relative uniformity of susceptibility to the BSE agent during passage through various animal species shows no phenotype changes in the agent on subsequent passage to mice (1). These transmission studies strongly suggested that the same major strain of the BSE was causative agent in all animal species tested (1, 2).

### **Comparative Study Using Scrapie-Resistant Sheep**

Based on their response to the scrapie challenge in England, sheep are divided into positive Sip genotype (susceptible, develop clinical disease) and negative Sip genotype (resistant, do not develop clinical disease) lines (34). Comparative experimental transmission studies using brains collected from natural scrapie sheep and BSE cattle in these two different Sip genotypes revealed that both susceptible and resistant lines of sheep developed the clinical disease with much shorter incubation periods through both i.c. and feeding with the BSE agent (1, 2, 26). This study demonstrated that the host gene, whether sheep were homozygous or heterozygous, played no role in the outcome of susceptibility when infected with the BSE agent.

Therefore, it is important to stress that breeding of the negative (resistant) lines of sheep might free English sheep flocks from Type I scrapie. As the resistant line of sheep are highly susceptible to the BSE agent from oral route, they, therefore, would get infected with more virulent strain Type II scrapie. The consequences would be that the BSE agent

would be present in sheep and cow, which suggests that man is at a greater risk of being infected than previously thought. Obviously, BSE spreading to other animal species would make eradication harder and more difficult without common Type I sheep scrapie, which has been acting as a natural vaccine for many farm animals including cattle. We can summarize the important points. The BSE agent is a highly efficient strain of TSEs. It has a unique stability when passed to other hosts. It can infect all mammalian species so far tested by oral route. The incubation period is preferentially being influenced by the strain of the agent rather than the host genetic makeup. Thus, it is unlikely that the PrP gene, with or without mutation, plays a major role in the incubation period and susceptibility.

### **Bovine Hypothesis**

The theoretical possibility exists that an unrecognized reservoir of a small number of subclinically affected cattle might have existed in low numbers whose carcasses had been used in the production of MBM. Previously, a single isolated suspected case of BSE was reported from the U.S. (63). There is also a possibility that the cattle might have been accidentally infected with a virus by error of some laboratory experiment or via a vaccine. An incidence in the 1930s had been documented when a vaccine against Louping-ill virus was prepared in England from sheep brains, spinal cord, and spleen. The vaccine was given to 18,000 sheep and subsequently, cases of scrapie were reported in sheep vaccinated with the vaccine (64).

The epidemiological records indicate that during the period of 1985 to 1989, incubation periods gradually got reduced from over 10 years to about 3 years (1, 2, 51, 52). It has been suggested that the epidemic arose as a result of the recycling of offal from cattle clinically or subclinically infected with BSE. In a laboratory, the procedure is called a serial passage and would show a reduction in the incubation period. Primary transmission from any species infected with TSEs to a different host species might affect only a minority of the inoculated animals and only after a prolonged incubation period. This is due to crossing a "species barrier," which may gradually disappear on subsequent passages in the new hosts, leading to shortening of the incubation period. This is where we should look for a real clue. A drop in the length of the incubation periods was obvious—it dropped in steps from about 10 years to 5 to 6, then settled at 2 to 3 years, but now it has increased again to over 5 years. This suggests that the agent was derived from another host species and has been pass several times in cattle.

### **Ovine Hypothesis**

To test the susceptibility of scrapie from sheep, cattle have been infected by i.c., intraperitoneal, intravenous, and oral dosing of brain homogenates from common Type I scrapie sheep (65). Although some of the infected cows developed a clinical disease, the symptoms were not typical of BSE. Furthermore, none of the calves revealed histopath-

ological spongy vacuolar changes similar to those seen in BSE.

It is important to note that none of the calves fed with large quantities of scrapie sheep brains developed clinical disease or showed any histopathological changes in the CNS. However, by comparison, all calves inoculated or fed with 1 g of BSE brain tissue developed typical clinical signs of BSE, and histopathological examination revealed much greater vacuolar degeneration compared with pathology in the calves inoculated with the scrapie sheep brain (27). To repeat, when sheep were infected with BSE, they developed clinical signs, including trembling and ataxia as seen in Type II scrapie.

In the same study, four calves, each inoculated with a different strain isolated from the TME agent, showed clinical signs approximately 15 months post-inoculation (27) with extensive vacuolar lesions. These findings suggested that the strain is similar both in TME, Type II scrapie, and BSE, but different from common Type I scrapie. These results conclusively show that Type I scrapie in sheep and BSE strains in cattle used in these experiments were different (27), but similar to Type II scrapie.

All evidence collected from strain typing studies demonstrates that the original BSE agent source is the rare Type II "trotting" scrapie of sheep, while the epidemic resulted from recycling of cattle remains to cattle. So far in the literature there are no reported cases of Type II "trotting" scrapie from the U.S. The absence of Type II scrapie from the American mainland may explain why there has been no BSE problem in the U.S., despite their having used similar rendering processes. It is probable that U.S. does have a small number of sheep with Type II "trotting" scrapie and just by good luck their carcasses have been used in mink feed, which may have caused small outbreaks of TME (66).

Clinically, Type II "trotting" scrapie is the same in its major symptoms as BSE, kuru, and human CJD cases infected with the BSE strain of the agent. These findings suggest that it is more than likely that sheep affected with Type II "trotting" type were part of a food stock on a ship to New Guinea that was consumed by the native people and caused kuru, while the cannibalistic practices among the native people helped the disease to spread further. Evidence linking BSE to Type II scrapie is ample; however, most of the experiments being done to demonstrate the origin of BSE are being carried out using Type I "itchy" scrapie. In summer 2000 at Birmingham, Dr. S. Prusiner stated that his colleague, Dr. Mike Scott, believes that sheep carry two strains of the agent: scrapie strain and the BSE strain (67).

### **BSE in Other Countries**

BSE in English cattle has brought about various implications and risks for many other countries. Most risk assessment studies are based on the information that has been provided by the English experience. Northern Ireland had over 1,000 cases since 1988. Epidemiological studies suggest that BSE in Northern Ireland did not arise from indig-

enous scrapie, but from MBM imported from England (68). It has been considered that most of the cases of BSE outside England have been due to exports of preclinically infected cattle or infected MBM.

Since the identification of BSE in the Republic of Ireland in 1989, the Department of Agriculture and Food has implemented an active surveillance program (69). A scheme of voluntary depopulation of the herds was introduced. Depopulation of affected herds formed a major difference of policy between England and the Republic of Ireland, the consequences of which reduced the chances of vertical transmission of BSE cases in Ireland compared with England.

In Switzerland, BSE was confirmed in Swiss dairy cattle in 1990. Thereafter, an extensive epidemiological investigation was carried out on each individual BSE case (10). Since the major factors contributing to BSE did not exist in the country, the occurrence of the disease in Switzerland was considered unexpected. The Swiss supported the English findings that BSE was a result of the exposure to contaminated MBM imported from England.

BSE cases recorded in Oman were traced back to cattle exported from England (70). Both cows were part of a consignment of 14 pregnant cows imported from England in 1985. Investigation suggested that the cattle were exposed to the contaminated foodstuffs in calfhood before the cows were exported from England. BSE has also been confirmed in France, Denmark, and the Falkland Islands. Most of these cattle were imported from England.

### **Possible Risk of Exposure to BSE in the United States**

The risk assessment on the possible occurrence of BSE in the U.S. has been carried out (71). Despite intensive surveillance efforts for the past 3 years, no variant of this disease has been detected in the U.S. cattle population (27). Between 1981 and 1989, 499 cattle were exported from England to the U.S. Of these, 343 have been slaughtered, 42 are missing, and the rest are under observation. Further, since September 1992, 1215 cattle brains with neurological or locomotory clinical signs compatible with BSE had been examined (72). No evidence of BSE lesions was found in these brains.

Until about 1980, it seems that very little MBM was fed to cattle in the U.S. Recent trends to feed nondegradable "by-pass" protein resulted in the use of more MBM, hydrolyzed feather meal, fish meal, poultry meal, and blood meal in cattle rations. These products are included in feed mainly for lactating dairy cows, which would indicate that their exposure would be from 2 to 5 years of age on average (71). As the incubation period for BSE is estimated to be 3 to 8 years (1, 2), only a small proportion of American cattle would have time to develop BSE before being culled. Exposure of cattle to MBM prepared from cattle subclinically infected with BSE could remain a threat in America for some years to come. In December 1989, American render-

ers decided to discontinue processing fallen and sick sheep, and some states have stopped rendering sheep material (71).

While BSE is not known to exist in the U.S., it is nevertheless important to compare epidemiologically regional differences among U.S. sheep and cattle. Key factors that should be considered include scrapie disease status, rendering processes, source of waste, and most important of all, existence and inclusion of Type II scrapie-infected sheep. Although MBM has also been prepared in many other countries, compared with England, very few, if any, BSE cases have been reported, particularly in the U.S. It is, therefore, important to establish whether Type II scrapie-infected sheep exist in the U.S. and other countries and whether cattle remains were included with sheep carcasses in the preparation of MBM, as appears to be the case in the English foodstuff.

Why did this disease appear only in the 1980's? Initially, the cause was attributed to the relaxation of laws concerning the heat treatment of animal MBM production at rendering plants while use of solvents was discontinued. One property of the TSEs agent had intrigued scientists for a long time, viz the survival of infectivity to boiling temperature. The question has to be asked: Can the TSE agent be destroyed by higher temperatures? Effects of heating at various temperatures have been tested by using the same infective scrapie inoculum from hamster throughout the experiment. After heating the brain homogenate at 121°C for 15 hr, eight hamsters were injected with a dilution of 1 in 1000. One out of eight experimental animals developed the disease in 285 days, while all hamsters developed the clinical disease in 90 days when injected with unheated sample in a dilution of 1 million (2). It is obvious that the incubation period increases as the infectivity is being destroyed by heat. Thus, heating does destroy the agent; however, it is not completely killed. In another study, a difference was observed between the susceptibility of drowsy strain and hyper strain when exposed to germicidal UV irradiation. The titre of drowsy strain was reduced by 99%, while the same amounts of UV irradiation had no effect on hyper strains of the TME agent. The variable effect of heating during MBM production would have selectively destroyed Type I scrapie, thus enabling Type II scrapie, a minor constituent of the total infectivity, to become a major component in the cause of BSE in epidemic form.

### **Role of Vertical Transmission in Spreading of BSE and Scrapie**

The pathogenesis of natural scrapie and BSE is poorly understood. However, vertical transmission is known to occur in sheep. In the dissemination of the disease from parent to offspring, the vertical transmission in the broad sense includes *in utero* exposure of fetuses. Since it is very difficult to distinguish between the two, the term maternal transmission has been used in many studies to include both prenatal and neonatal infection (73). Indirect evidence of *in utero* maternal transmission has come from a number of

studies. Experimentally infected pregnant ewes produced a number of unexpectedly early-infected cases in their offspring (74, 75). Some earlier attempts to detect the scrapie agent in fetuses of affected sheep had failed (5). However, in later studies, Hourrigan (76) found evidence of the scrapie infection in the fetus of an affected sheep. The scrapie agent has been detected in a placenta from an infected sheep (77) and also in various parts of the ewe's reproductive tract (78). What role the placenta and reproductive parts of infected females play during gestation is not known. However, the infected organ would appear to form a regular source of extraneural infection in the environment.

Since natural scrapie has never been recorded in the Sip pApA genotype of NPU Cheviot sheep, they were used as surrogate ewes for embryo transfer technique to investigate maternal transmission of scrapie in sheep and BSE in goats (79). Donor ewes were infected with the scrapie agent and were artificially inseminated 6 months later with semen from an uninfected but scrapie-susceptible ram. The embryos were harvested 5 to 6 days post-insemination and were transferred by laparoscopy into surrogate Sip pApA genotype ewes genetically selected for low susceptibility to scrapie. When the lambs were born, they were kept and grazed on pasture never previously exposed to scrapie-infected or parturient sheep. Ten of the 26 lambs born developed the clinical disease along with all donor-infected sheep, while the surrogate ewes remained healthy. These studies conclusively reveal that donor embryos were all infected prior to transfer to the surrogate ewes. In England, since calves had been fed with contaminated MBM starting from 4 to 6 weeks of life and throughout their life, it is more than likely that when they become pregnant, maternal transmission of embryos will take place. Based on these results, it is very hard to predict what proportion of calves born of affected cattle will develop clinical BSE.

Wilesmith in 1990 (80) suggested that BSE agent will clear itself from the cattle population by breeding. However, to address this question of vertical transmission, MAFF housed 630 cattle in isolation on four farms. Half the calves (315 in Group 1) were taken from cattle who were known to have died from BSE. The other half (315 in Group 2) were selected from the same farms. At the time of selection, parent cows were healthy, although no one knows whether they were incubating the disease, and some have suggested that both groups were fed ruminant protein and may have been exposed to infected feed. However, 42 of the 273 cows in Group 1 and 14 in Group 2 developed BSE. Further, diagnostic lesions were observed in the brains of four others (the group is unknown) that were killed when they were 7 years old without clinical BSE having been suspected. This provides good evidence of vertical transmission.

### **Control and Eradication of BSE**

Cows are herbivores, which were turned into carnivores, and most importantly, into cannibals. It is believed that the cannibalistic ritual from man to man was the sole

source of the kuru epidemic in Papua New Guinea (81). Evidence presented in this review suggests that sheep affected with Type II "trotting" scrapie caused kuru, while the epidemic was then caused by cannibalistic rituals. A ban on cannibalistic rituals from man to man appears to have eliminated kuru. It was considered that a ban on the feeding of MBM would probably be the single most effective measure to control BSE. A ban on the use of MBM to cattle was introduced in July 1988. However, in late 1996, after a delay of 8 years, a similar ban on the use of MBM was introduced for other farm animals, pigs, poultry, and fish. The continued epidemiological studies have revealed a drop of BSE cases in recent years.

In 1999, 94% of BSE cases being confirmed were in cattle born after a ban on the use of MBM was imposed in England in July 1988. Some farmers have reported that incidences of neurological defects have increased in calves born since the origin of BSE. Some farmers suspect that these could be early cases of BSE, as seen in a child born of a CJD mother. However, MAFF has not carried out histopathological examination of brains of calves with neurological defects. It is important to stress that cattle for beef are normally slaughtered before 18 months of age, while most recorded cases of BSE have occurred in cattle over 48 months old. On the assumption that asymptomatic cattle under the age of 30 months would not be able to pass on infection, MAFF agreed early in 1996, as a safety measure, that both beef and dairy cattle over 30 months old, when slaughtered, had to be turned into MBM and burned. On average, over 800,000 cattle have to be slaughtered each year for this purpose and this turns them into 400,000 tons of MBM to be burned. This is a very costly affair. To solve this problem, the surplus animals should be culled at a much earlier age. It may not reduce the number of animals slaughtered, but it will reduce the weight of the dead carcasses to be handled, making a big difference in the amount of MBM and compensation paid. It should be pointed out, however, that because of vertical transmission of BSE, culling cattle at any age is not sufficient to eradicate BSE.

### **Prospects for a BSE Vaccine**

A phenomenon analogous to "interference" has been demonstrated by the inoculation of mice, first with the long-incubation (600 days or more) strain of the scrapie agent followed by inoculation a few weeks later with a short-incubation (150 days) strain of the scrapie agent into the same mice. Inoculation first with the "long" incubation strain inhibits replication of the "shorter" strain, while inoculation first with the "short" incubation strain inhibits replication of the "long" incubation strain (1, 2, 62, 82). Phenomenon of interaction and interference between different strains of scrapie are well established, which forms the basis for developing a vaccine against BSE (1, 2, 62, 82). At the 18th International Congress of Biochemistry and Molecular Biology in Birmingham in 2000, Dr. S. Prusiner stated that his colleague, Dr. Mike Scott, also believes that

the scrapie strain is somewhat dominant, preventing the BSE strain from infecting cattle and people when both are present (67). In other words, the scrapie strain acts as a vaccine against the BSE strain.

As discussed before, experimental feeding of cattle and mink with scrapie sheep brain tissues does not lead to development of the clinical disease, even after they have eaten large quantities of contaminated brain tissue (27). Comparative studies using mink and cattle injected with scrapie also showed very poor uptake, and those fed with the scrapie sheep brains do not develop clinical or pathological disease. On the contrary, mink and calves fed or injected with BSE always develop the disease. The phenomenon of "interference" should, therefore, be further explored. It would be of great importance to know if this scrapie strain of the agent blocks the BSE agent when mink and cattle prechallenged with scrapie are then challenged with BSE. Development of such a vaccine would stop horizontal transmission of BSE in cattle and help to eradicate BSE.

### The Public Health Implications of BSE

Scrapie has been common in sheep in England since the 1750s and, of course, sheep have been eaten with no apparent health hazard (1, 2, 29). Type I scrapie is common in sheep and is the only firmly established natural reservoir of TSE infections in animals. Sheep and cattle have often shared the same grazing fields without the cattle being affected with BSE. To assure the public, one major assumption has been made: that BSE is the same strain as found in the scrapie sheep. BSE has occurred through the crossing of the "species barrier" from scrapie sheep to cattle. It has been all right to eat scrapie sheep before, therefore, what harm could come from eating cattle with BSE? However, as discussed before, the origin of the BSE strain is from the rare Type II "trotting" scrapie as is obvious from the published scientific literature (2, 55, 56).

In the past and even now, asymptomatic cattle are considered to contain a low titre of infectivity and, therefore, the assumption has been made that they are not infectious or dangerous to human or animal health. Scientifically, a low titre of infectivity for the TSE agent suggests that it is infectious enough to infect a small number of humans and the clinical disease would develop after a prolonged period of incubation. The clinical disease appearance was used as an index of infectivity and only then was it considered unsafe to eat. It was also assumed that only tissues in which PrP plaques could be found were the sources of infection.

Along with many other investigators, we have suggested that the prion protein is not the infective agent, but we consider the agent to be a virus with DNA. This DNA is closely associated with a PrP/SAF that lies behind the problem (22–25). To understand why humans were considered to be at a much higher risk with BSE compared with scrapie in sheep, we have to examine strain differences.

Possible routes of the scrapie infection from sheep to humans remain a mystery. The disease is readily transmitted

experimentally by the oral administration of scrapie-infected sheep tissue to sheep and goats (77, 83), including the fetal membranes from an infected animal (83). In sheep, the oral route seems to be one of the important routes of infection (84). However, the epidemiological evidence indicates a predominantly maternal pattern for the transmission of the infection (85). There are a number of previous epidemiological studies that failed to establish an epidemiological connection between scrapie sheep with the human diseases, kuru, CJD, GSSS, and other animal diseases (86).

The most important feature of serial passage in cattle has been the selection of the single most virulent Type II "trotting" strain, now termed the BSE agent. This strain has a relatively short incubation period and high efficiency of primary transmission to most animal species, including farm animals, which form part of the food chain for humans. The most worrying feature of the strain is that it readily infects by feeding it to a variety of animal species. If the BSE agent can infect so many animal species in such a short time, humans, like other ruminant and nonruminant species, are also at great risk. These features have raised the question as to whether or not there are genetic host differences in humans that will prevent them from being infected by the TSE agent.

In order to find out whether BSE could be transmitted to humans, a great deal of emphasis has been paid to transgenic mice experiments. Collinge *et al.* (87) inoculated non-mutant (wild-type) mice and various transgenic mice carrying human PrP (HuPrP) gene with the BSE and CJD agent. The survival time of 250 to 300 days of HuPrP transgenic mice inoculated with the CJD agent was found to be significantly shorter than those of wild-type mice (480 days or more). However, in the same study, mice inoculated with the BSE agent revealed no significant difference in the survival time (450–520 days) between transgenic mice expressing both human and mouse PrP and parent strains from which the transgenic mice had been produced. Transgenic mice expressing only HuPrP who exhibited shorter incubation period with the CJD agent were inoculated with the BSE agent. These mice did not develop disease by 268 days after they were injected with the BSE agent; therefore, the authors concluded that humans might not be at risk of contracting BSE as previously thought. Transmission to wild-type strains of mice has resulted in clinical disease after 600 to 700 days; it was premature, while the incubation periods could take up to 2 years, to conclude from this experiment that BSE was not a risk to humans (88). Eventually, these mice developed the clinical disease. It is important to realize, in terms of percentage, that the transgenic mice are no more susceptible than nontransgenic mice; that some transgenic mice develop the disease with a reduced incubation period, while others have a prolonged incubation period; and that the number of mice used in these experiments did not exceed more than a handful. However, millions of people have been exposed not once, but over and over, to the BSE agent. Many will live more than 60 years. In other

words, the transgenic mice experiment is only significant if it reports in the positive. This should be very obvious from earlier mice experiments in which the deliberate contamination of human growth hormone with the scrapie agent showed that the production process could remove contaminants even when present at high concentration (89). The growth hormone from human pituitary glands was considered safe for human use, but ultimately it was found not to be safe.

After many years of research, we still lack the basic understanding on the main issues: the route of infection, the source of transmission, the causative agent that makes the disease contagious, and cases of vertical transmission and horizontal transmission. Another misapprehension is that only cows with full-blown BSE can contaminate farmland with their dung and urine (2). This cannot be true. If animals had been eating contaminated MBM rations, it would be excreting any undigested disease agent from day one. We need to know answers to all these questions and many more, especially the nature of the agent, its diagnosis, and its control.

Even before BSE appeared, it was known from Japanese and American studies since the 1980s that mice develop the disease when inoculated with blood of CJD patients, both during the incubation period and during the clinical phase of CJD (90–93). Klein *et al.* (90) and Tateishi (91–93) independently transmitted the disease in mice from crude suspension made in normal saline of the brain, cornea, and untreated cerebrospinal fluid (CSF), blood samples, and urine from a patient infected with CJD. These studies conclusively confirm the presence of the CJD agent in blood, regardless of the strain (29). Recently, Houston *et al.* (94) confirmed that it is possible to transmit BSE to sheep by transfusion with whole blood taken from another affected sheep. The donor sheep developed BSE 629 days after it was fed with 5 g of BSE-affected cattle brain, while the blood used was taken 318 days after the oral challenge, roughly half way through the incubation period during the symptom-free phase of an experimental BSE infection. Recently, Narang reviewed clinical histories of 27 CJD cases that resulted in death during 1989 to 1996. Out of those 27 cases, six were regular blood donors, while one was a blood recipient. A comparative histopathological examination of the brain sections of the recipient of blood, human growth hormone, and those infected with the BSE strain cases revealed extensive vacuolation of the cerebellum in all cases with numerous PrP positive plaques. In the brains of the blood recipient and human growth hormone, PrP plaques were much smaller in size compared with the patients infected with the BSE strain of the agent (32). This identical size and distribution of PrP plaques in the recipient of blood and human growth hormone brains and different from those infected with the BSE agent demonstrates that the source of infection was a result of accidental inoculation of the contaminated product. It is obvious that no blood or blood product from any CJD case, symptom-free phase, or with

symptoms can be regarded as infection free. There is an urgent need to screen all blood donors for CJD.

Since red meat contains a fair amount of blood, obviously it may be carrying the BSE agent. Although titres might be low, the amount consumed by each person is high and is therefore a potential source of infection. It is crucial to find out if red meat and milk contain the infective agent. This can be achieved by feeding them to mink, which are very susceptible to the BSE agent by the oral route and not to the scrapie agent.

The public health implications of BSE are particularly difficult to address. One should compare these implications with the incidence where human growth hormone prepared from human pituitary glands had been used. The number of CJD patients treated with human growth hormone appeared after a long incubation period and their number increases every year. Because of the long incubation period, we will not know for some time, maybe a few years, as in the case of growth hormone, how many humans are subclinically affected and how many of these will develop the clinical disease at some later stage.

The possibility of risk to humans has further increased as the BSE agent has spread to other animal species. Its control has to be seriously considered. However, there appears to be a blessing in disguise, based on the known “interference” phenomenon that exists within the TSE agents. If humans have eaten scrapie sheep strain, the BSE agent will not be able to establish in these exposed cases (2, 29). Most of the older people may be perhaps protected by having eaten scrapie-infected lamb, while the young are more vulnerable because they might not yet have consumed scrapie-affected lambs.

## In Conclusion

Spongiform encephalopathies in animals or humans are slowly developing infectious diseases of the central nervous system, and their incubation periods are dose dependent. Over 20 strains of the infectious agent in scrapie of sheep have been identified. However, based upon clinical signs, there are two distinct syndromes, both of which have been called scrapie. In the Type I scrapie, sheep express itchiness and lose their wool. In the Type II scrapie, sheep express trembling ataxia and a “trotting gait.” Despite lack of compelling evidence, the origin of BSE was considered to be due to feeding cattle with meat and bone meal prepared from waste remains of sheep-infected with the Type I scrapie. The “cannibalistic” practice of feeding cattle has resulted in the selection of a more virulent minor strain from the Type II scrapie sheep with trembling ataxia. Evidence suggests that Type II is the cause of BSE and kuru. The BSE strain has shorter incubation periods and appears very efficient in infecting other species by the oral and intracerebral routes. A single infected BSE cow, whether expressing symptoms or not, entering the human food chain with the “agent” active and finding suitable infection courts could potentially infect a large number of humans. Risk assess-

ments for humans are difficult to make, while consequences could be grave.

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