2 Background

This section provides background for the analysis described in this report. Section 2.1 outlines the characteristics of transmissible spongiform encephalopathies (TSEs), the class of diseases to which BSE belongs. Section 2.2 reviews the hypotheses advanced for the origin of the BSE epidemic in the UK. Section 2.3 describes potential pathways by which BSE could be introduced into the U.S. Finally, Section 4 reviews regulatory actions taken by governments around the world to slow the spread of this disease.

2.1 Overview of Transmissible Spongiform Encephalopathies (TSEs)

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a family of rare, slowly progressive, and uniformly fatal neurodegenerative disorders that affect humans and animals. All of these diseases have incubation periods of months to years between infection and the onset of clinical signs. The prevailing hypothesis is that these diseases are caused by novel agents called prions. In humans, prion diseases may present as genetic, infectious, or sporadic disorders, and all involve the modification of the prion protein. Known TSEs include:

- Creuzfeldt-Jakob disease (CJD), Kuru, Gerstmann-Straüssler-Sckeinker (GSS), and Fatal Familial Insomnia (FFI) in humans;
- Scrapie in sheep and goats;
- Transmissible Mink Encephalopathy (TME) in mink;
- Chronic Wasting Disease (CWD) of deer and elk; and
- Bovine Spongiform Encephalopathy (BSE) in cattle.

Other examples, such as Feline Spongiform Encephalopathy (FSE), are thought to be the result of cross-species transmission of a TSE (BSE in the case of FSE). Variant CJD (vCJD) is a newly discovered TSE of humans and is likely the result of exposure to the BSE agent.

The first records of TSEs date back to the early 18th century (Stockman, 1913, Brown and Bradley, 1998), with the mention of scrapie in sheep. The name of the disease reflects its associated clinical signs, including the tendency of the sheep to scrape off their wool on fences or

other objects. Experimental transmission of scrapie to other species, such as mice and goats, demonstrated that the disease was transmissible and had a very long incubation period (Cullie and Chelle, 1936, Cullie and Celle, 1939, Pattison et al., 1959).

During the 1950s, many scientists became interested in Kuru, a fatal disease that affected the Fore population of Papua, New Guinea. Kuru is characterized by neurologic signs and neuropathologic changes similar to those of scrapie (Zigas and Gajdusek, 1957, Klatzo et al., 1957, Alpers, 1970). These similarities were pointed out by Hadlow in 1959, who suggested that Kuru might also be transmissible to other animals (Hadlow, 1959). Subsequently, Gadujsek and his colleagues succeeded in transmitting Kuru to chimpanzees (Gajdusek et al., 1966). This experiment supported the hypothesis that Kuru is transmitted by an infectious mechanism (*i.e.*, ritualistic cannibalism). Later, other spongiform encephalopathies were found to be transmissible, including CJD, FFI, TME, CWD, and BSE. To date, most of the experimental data on TSEs comes from studies of CJD and scrapie. More recently, BSE has become an area of active research.

The specific agent responsible for TSE diseases has not been identified with certainty, but the leading theory suggests that the etiologic agent is an abnormally configured protein normally encoded by the host (prion protein or PrP) (Bolton et al., 1982, Prusiner, 1982, Prusiner, 1994, Prusiner, 1998). Normal prion protein (PrP^{c}) is soluble in detergents and has a predominantly α-helical structure. In contrast, abnormal PrP (PrP^{sc}) is insoluble in detergents, relatively resistant to proteases, and has a predominantly β-sheet secondary structure. Although still a matter of controversy, PrP^{sc} appears to accumulate in an infected host and eventually cause disease (Bueler et al., 1993, Manson et al., 1999, Hsiao et al., 1991, Telling et al., 1995, Hill et al., 2000). Deposits of PrP^{sc} in tissues are associated with the presence of transmissible infectivity (McKinley et al., 1983). Additionally, PrP^{sc} is the only molecular marker specific for TSE infections. Spongiform degeneration, neuronal vacuolation, and gliosis appear to be associated with abnormal PrP deposition. Remarkably, TSE infection has been reported in the absence of detectable PrP^{sc} (Lasmezas et al., 1997) and PrP^{sc} formed *in vitro*, by conversion of PrP^{c}, has not yet produced disease in animal bioassays (Hill et al., 1999). The etiologic agent is not inactivated by treatments that usually destroy bacteria and viruses (Kimberlin et al., 1983, Taylor, 1991b, Taylor, 1991a, Taylor, 1993). No immune response to the agent has been detected.

An alternative hypothesis to the prion theory, referred to as the virino model, proposes that the agent consists of a small nucleic acid that acts as an informational molecule, and that this molecule is protected by the host PrP (Dickinson and Outram, 1988). Despite several attempts (Borras and Gibbs, 1986, Duguid et al., 1988), no exogenous nucleic acid has been identified in experimental TSE. The virino model suggests different genetic "strains" of the agent are responsible for the phenotypic variability in the disease. The protein only (prion) hypothesis proposes that conformational isoforms of PrP are responsible for such variability. Another theory proposes that TSEs are caused by conventional viruses (Diringer et al., 1994, Manuelidis et al., 1995). However, no infection-specific nucleic acid has yet been detected.

The mechanisms by which infection occurs for most naturally occurring TSEs are uncertain. Different animal TSEs appear to be passed in part by lateral transmission and perhaps by maternal transmission to offspring in natural settings. The human spongiform encephalopathies are considered to be either sporadic, inherited, or acquired by an infectious mechanism (Masters et al., 1978, McLean et al., 1998, Hsiao and Prusiner, 1990, Brown et al., 1994a, Will et al., 1996). Finally, there is evidence that for some TSE diseases, susceptibility has a genetic component (Poulter et al., 1992, Carlson et al., 1994, Hunter et al., 1996, Hunter, 1997, Hunter et al., 1997a, Bossers et al., 1997, Goldmann et al., 1996).

The remainder of this section has three parts. Section 2.1.1 discusses the means by which TSEs are passed from one animal to another, and perhaps from animals to humans. Section 2.1.2 introduces the concept of the "species barrier," a phenomenon that makes passage of a TSE from one species to another far less "efficient" (and hence less likely) than passage between animals of the same species. Finally, Section 2.1.3 discusses susceptibility, *i.e.*, the tendency for some animals to be more likely than others to become infected following exposure to the infective agent.

2.1.1 Transmissibility

TSE diseases can be passed from an infected individual to others under only certain conditions. While the potential for natural transmission has been demonstrated only for some TSEs, transmission in an experimental setting has been demonstrated for most.

Transmission of a TSE disease from one human to another appears to be limited to cases of "iatrogenic transmission," associated with surgery, use of cadaveric hormones, and ritualistic cannibalism. Iatrogenic transmission is the only known route of transmission for CJD. Documented cases have involved the use of contaminated silver electrodes used for stereotactic electroencephalography, the use of contaminated neurosurgical instruments (Collinge and Palmer, 1997), and the use of contaminated tissues in transplant procedures (cornea, dura mater). The use of contaminated hormones preparations (growth hormone or gonadotropin prepared from cadaveric pituitary glands) has been linked to transmission of TSEs in humans. Kuru has been transmitted from person to person as the result of ritualistic cannibalism in the people of Papua, New Guinea. In this case, the most likely route of exposure was *via* ingestion, although transdermal or mucous membrane exposure cannot be ruled out.

Transmission of TSE diseases from one animal to another of the same species in the absence of experimental intervention has been extensively documented in the case of sheep-borne scrapie (Hadlow et al., 1982). The mechanism by which other sheep in the same flock become infected appears to be associated with exposure to infected placenta (Race et al., 1998), although other routes of exposure may also play a role in transmission. In sheep, transmission has also been linked to the use of vaccines (Gordon, 1939, Gordon, 1946, Gordon, 1959, Agrimi et al., 1999). Natural transmission has also been identified in CWD (Williams and Young, 1982). In the case of CWD, it has been postulated that transmission is caused by pasturing on ground occupied by infected animals (Miller et al., 1998). Presumably, some long-lived agent in the environment can pass the disease between individuals (Skarphedinsson et al., 1994, Sigurdson, 1991). Naturally occurring mutations capable of causing the disease have not been identified in animals (Chesebro, 1999).

Several species and animal breeds have been used as experimental models for TSEs, including mice, hamsters, and non-human primates. Results from these experiments indicate that natural (oral) transmission is substantially less efficient than transmission *via* intracerebral (i.c.) injection, the procedure usually used for transmission in experimental models. For example, in the case of BSE, transmission *via* oral ingestion is as much as 100,000 times less efficient than i.c. injection (for a review of the literature, see (SSC, 2000b)). Experimental data using a TSE mouse model indicate that intravenous injection produces disease five to seven times less efficiently than i.c. injection (Brown et al., 1999). Finally, intra-peritoneal (i.p.) administration of

infectivity is estimated to be 100 times less efficient than i.c. transmission (Kimberlin and Walker, 1988).

2.1.2 The Species Barrier

Interspecies transmission of TSEs is mitigated by a so called "species barrier". This barrier represents the decreased efficiency with which TSEs are passed from one animal to a second animal of a different species, compared with the efficiency with which the TSE is passed among animals of the same species. That is, a much greater amount of infective material is necessary to infect an animal from a different species than is needed to pass the disease to an animal of the same species. The species barrier is also associated with an increase in the disease's incubation period (*i.e.*, the delay between exposure to the agent resulting in infection and the manifestation of disease). In some instances the species barrier seems to confer complete resistance to transmission. It is at least conceptually possible that an animal failing to develop the disease following cross species challenge would become infected if administered a sufficiently large dose of infectivity, or would manifest clinical signs of disease if it somehow lived longer than the incubation period associated with the species barrier (Hill et al., 2000).

Although transmission of a TSE from one species to another may be less efficient than the transmission within the same species, once it occurs, the TSE may become "adapted" to the new host. Because it has adapted to the new species, it can be transmitted more efficiently among members of that species, and the incubation period becomes shorter and less variable. For example, when scrapie is transmitted experimentally from one species to another, the incubation period is usually longer in the first passage than that seen in subsequent passages within the new species (Dickinson et al., 1976).

The species barrier probably reflects some combination of factors including differences between the donor's and recipient PrP. Scrapie studies conducted in mice, rats, and hamsters demonstrate the presence of a species barrier. These findings include pathogenesis differences between the first and subsequent passages in the new species, and how rapidly the transmitted strain replicates in the new host (Kimberlin et al., 1987, Kimberlin and Walker, 1989), and others.

The response of some TSEs exhibits heterogeneity within a species, a characteristic that appears to be due to the existence of different strains of the agent. Strains are distinguished by

highly replicable differences in the incubation period, neuropathology, and host range (Fraser and Dickinson, 1968, Bruce et al., 1989). CJD, scrapie, TME, and CWD show strain diversity, while BSE appears to be a single, stable strain (Bruce et al., 1994, Bruce et al., 1997). vCJD (*i.e.*, the new form of CJD related to exposure to the BSE agent) does not demonstrate morphologic strains (Will et al., 1996, Hill et al., 1997, Bruce et al., 1997, Scott et al., 1999).

Recipient characteristics also affect the efficiency with which TSEs are transmitted across species. Some species, such as rabbits or chickens, do not develop disease when challenged with specific TSEs, while other species do. It has been postulated that the similarities between the PrP structure between the donor and the recipient explain the differences in transmission efficiency (Priola et al., 1994, Raymond et al., 2000).

Because the presence of a TSE agent is often assessed by inoculating a test species (*e.g.*, mice) with the suspect material, the species barrier compromises the sensitivity of these bioassays. Cattle-to-cattle transmission of BSE by the intracerebral route is known to be 1,000 times more efficient than cattle-to-mouse transmission by the same route (MAFF, 2000b). It is often assumed that the species barrier decreases transmission efficiency by a factor of between 1 (no decrease) and 1,000 (Det Norske Veritas, 1997). The assumption that the species barrier is 1 (*i.e.*, that there is effectively no species barrier) is considered to be a worst-case scenario. In an opinion on the species barrier for transmission of BSE from cattle to humans, the EU Scientific Steering Committee suggested that plausible values for the impact of the species barrier on transmission efficiency range from between 1 and 100,000 (SSC, 1999a). This range was later updated to between 10 and 100,000 (SSC, 2000b). However, the committee concluded that it is impossible to estimate the true value for BSE species barrier between cattle and humans within an order of magnitude given current knowledge (SSC, 2000b).

2.1.3 Susceptibility

"Susceptibility" refers to the likelihood of becoming infected following a specific exposure to the infective agent. Susceptibility to TSEs appears to depend on specific interactions between the agent, the host, and the environment (*e.g.*, animal age, PrP primary structure of the host, PrP characteristics of the recipient animal, route of exposure, and dose of agent).

Mutations and polymorphisms of the PrP gene are associated with many TSEs in humans, sheep, mice, and possibly elk. Humans and sheep are the two species for which spongiform encephalopathies apparently occur naturally and in which there are recognized genetic components that predispose individuals to disease. In the case of scrapie, there is evidence that the disease does not develop spontaneously, but instead requires exposure to an infective agent (Hunter et al., 1996, Hunter and Cairns, 1998). In the case of human disease, studies conducted using in transgenic mice that over express mutant PrP [P101L (corresponding to PrP P102L in humans)] have shown that Gerstmann-Straüssler Skeinker might be a genetically induced illness (Hsiao et al., 1991, Hsiao et al., 1994). However, recent research using transgenic mice that are normal expressers of mutant PrP failed to demonstrate development of the spontaneous TSE (Manson et al., 1999), suggesting that the mutation may increase susceptibility to infection (Weissmann and Aguzzi, 1999, Manson et al., 1999), rather than cause the disease on its own. In contrast, in cattle, susceptibility to BSE has not yet been shown to be associated with polymorphism in the PrP gene (Hunter et al., 1994).

An important physiologic factor that is likely to affect susceptibility to infection is the age of the animal. For example, young cattle are estimated to be ten times more susceptible than adults, with data well described by a model that assumes susceptibility declines exponentially with an annual rate constant of 0.85 after the age of four months, with susceptibility ultimately declining to 10% of its peak value (Koeijer et al., In press). An alternative estimate computed by back calculating a model of the UK BSE epidemic suggests that susceptibility peaks at 1.31 years and decreases in the following years (Anderson et al., 1996). Other investigators estimated that susceptibility in cattle peaks between 0.5 to 1.5 years of age (Woolhouse and Anderson, 1997). Age-related susceptibility is hypothesized to be associated with permeability of the intestine to large proteins and with the development of the Peyer's Patches (PP). The PP seem to play a role in the pathogenesis of the prion diseases and to influence the susceptibility of the animal to infection. For example, in sheep, the ileal PP are shown to be more active and to be largest when the animal is around 2-3 months old and to disappear when the animal reaches an age of one and one-half years (Griebel and Hein, 1996). The appearance of the PP in sheep appears to coincide with the period of greatest susceptibility of sheep to scrapie (Hadlow et al., 1982, Andreoletti et al., 2000).

Age-related susceptibility may be an important factor in understanding BSE transmission because potential exposure to BSE-contaminated feed (see Section 2.2 and Section 3.1.1.2 below)

can also change with age. The ages at which animals are exposed depends on when they receive feed with protein supplements, something that may vary from country to country. For instance, Meat and Bone Meal (MBM) was used in the formulation of "least cost" calf starter rations in the UK during the period of 1970-1988 (Horn et al., 2001), leading to BSE exposure when animals are most susceptible to disease.

The remainder of this discussion addresses susceptibility issues specific to sheep, humans, bovines, and cervids in turn.

Sheep

In sheep, polymorphisms identified at codon 136, 174 and 171 of the PrP gene play the largest role in variations in the development of natural scrapie. The clinical and pathological variations of the disease are a direct result of host-agent interaction. Holding both dose and route of transmission fixed, the transmission of scrapie depends on the homology of the donor's PrP and the recipient's PrP. Some aspects of the pathogenesis can differ depending on the interaction of agent strain, host genotype, route of infection, and dose of the agent.

Naturally infected sheep of a number of breeds in the US, UK, Europe, and Japan carry valine at codon 136 ($VV_{136\ or}\ VA_{136}$) or glutamine at codon 171 (QQ_{171}) (Laplanche et al., 1993, Westaway et al., 1994, Belt et al., 1995, Ikeda et al., 1995, Hunter et al., 1993, Hunter et al., 1994) of the PrP gene. There has only been one report of scrapie-affected Suffolk with arginine homozygosity at codon 171(RR_{171}) and four reports of scrapie affected Suffolk with glutamine/arginine heterozygosity at codon 171 (QR_{171}) (Ikeda et al., 1995, Hunter et al., 1997b). Scrapie strains can be distinguished by biological parameters such as the incubation period, lesion profile, and amyloid plaque production (Dickinson and Meikle, 1971, Dickinson and Outram, 1988, Bruce and Fraser, 1982, Bruce et al., 1991, Bruce et al., 1997).

There has been some debate as to whether naturally occurring scrapie is a purely genetically-induced disease (Ridley and Baker, 1996) or if PrP genotype merely influences susceptibility following exposure to an infectious agent. The current consensus rules out the hypothesis that scrapie is a purely genetic disease (Hunter and Cairns, 1998, Hunter, 1998) and suggests that susceptibility and exposure are both necessary for the development of the disease.

Sheep and goats have been shown to be susceptible to the development of BSE following experimental exposure (i.c. and oral) (Foster et al., 1993, Foster et al., 1996, Bruce et al., 1994). Different PrP genotypes have different incubation periods (Foster et al., 2001) following BSE exposure. Currently, there is no evidence that sheep and goats can develop the disease after exposure to feed supplemented with contaminated animal protein.

Humans

In humans, polymorphisms in the PrP gene influence susceptibility to sporadic, inherited, or infectious forms of prion diseases. There are two common forms of PrP in humans with either methionine (M) or valine (V) at residue 129. The population is comprised of homozygous M, heterozygous M-V, and homozygous V. In Caucasians, 51% of the population are heterozygous while 38% are methionine homozygous; the least common genotype is valine homozygous (11%). Variability of spontaneous CJD seems to be associated with physicochemical properties of PrP^{sc} in conjunction with the *PRNP* (human prion protein gene) codon 129 genotype (Parchi et al., 1999). In Kuru patients, homozygosity at residue 129 (particularly for methionine) was associated with an earlier age at onset and a shorter duration of illness than was heterozygosity at residue 129, a finding that probably reflects different disease incubation periods (Cervenakova et al., 1998).

Homozygosity at residue 129 appears to increase susceptibility to TSE disease in humans. Cases of sporadic CJD are usually homozygous at residue 129 (Palmer et al., 1991). Individuals with CJD caused by exposure to contaminated human pituitary hormone have an elevated prevalence of homozygosity at residue 129 for valine (Collinge et al., 1991). In familial TSEs, polymorphism at codon 129 appears to influence the age of onset and the duration of the disease (Dlouhy et al., 1992). To date, all vCJD patients have been methionine homozygous (M-M) at residue 129 (Will et al., 2000, Ironside et al., 2000).

Bovines

Investigators have identified polymorphisms in the PrP gene in British cattle, Belgian cattle, and US cattle (for review refer to (SSC, 2000b)). There are two major polymorphisms in the region of the PrP gene: 1) the HindII restriction site, and 2) differences in the number of copies (5 or 6) of an octapeptide repeat sequence (Goldmann et al., 1991, Hunter et al., 1994).

Hunter et al. (1992) showed that there were no differences among breeds in the age of onset of BSE. Nor did the number of PrP octapeptide copies influence age of onset. The absence of an association between PrP polymorphisms and BSE onset age may indicate that BSE incidence is associated with an "undiscovered" polymorphism of the PrP gene. It could also mean that there are other mutations that influence gene expression and potentially disease onset. Alternatively, there may be only one predominant form of cattle PrP, and if this predominant form were the allele that conferred susceptibility, most cattle would be genetically susceptible. In this case, the dose and route of exposure (assuming there is only one strain of BSE) determine whether disease results.

Findings from the pathogenesis and the attack rate experiments (Wells et al., 1998, Wells et al., 1999), in which animals were exposed to high levels of infectivity (1 to 100 times greater than most cattle would have received naturally), indicate that most of the cattle challenged either orally or parenterally succumbed to disease. These results suggest that differences in susceptibility between animals may not exist, or may not be important. Alternatively, the exposures may have been so high that they overwhelmed any differences in susceptibility. Overall, it appears that if animals are exposed to high doses of the BSE agent early in life, they will be very likely to develop disease.

Cattle have been shown to be partially susceptible to naturally induced scrapie but only following intracerebral injection of infectious material (Gibbs et al., 1990, Clark et al., 1995, Cutlip et al., 1994, Cutlip et al., 1997, Cutlip et al., 2001). Studies done in the U.S. showed that cattle orally exposed to North American scrapie remain normal for eight years following exposure (Cutlip et al., 2001). Research on cattle orally exposed to UK scrapie is ongoing (Linda Detwiler, personal communication). Currently, there are no available data indicating how genetics might influence bovine susceptibility to scrapie.

Cervids

In a study of Rocky Mountain Elk, O'Rourke et al. (O'Rourke et al., 1999) found that animals with CWD had an elevated prevalence of homzygosity for methionine at codon 132 of the PrP gene. This finding applied to both farmed and free-range animals.

2.2 The Origin of the BSE Epidemic in the UK

In 1986, a bovine spongiform encephalopathy was first confirmed in the United Kingdom as the result of routine animal disease surveillance. This section focuses on theories advanced to explain the origins of the subsequent epidemic. Since the beginning of the epidemic, over 178,400 cases have been confirmed on 35,275 farms. In addition, cases have been observed in Northern Ireland, the Republic of Ireland, and in other European countries (OIE, 2000). Mathematical modeling suggests that the epidemic probably started in the UK between 1981 and 1982 (Wilesmith et al., 1991, Wilesmith et al., 1992, Wilesmith, 1994). The epidemic peaked at the end of 1992-1993 when the incidence reached approximately 3,500 confirmed cases per month. Although the origin of the BSE epidemic remains controversial, there is little doubt that it was maintained by the recycling of bovine materials in the bovine feed chain (Kimberlin and Wilesmith, 1994, Wilesmith et al., 1991, Wilesmith et al., 1992, Wilesmith, 1994, Nathanson et al., 1997).

Although the effectiveness of the feed ban and other measures at reducing the incidence of BSE in the UK sheds light on the progress and amplification of this epidemic (Section 2.4.2), its precise origin remains uncertain. The most prominent theory hypothesizes that BSE occurred when the scrapie agent, present in rendered proteins used in feed, overcame the species barrier to infect cattle (Section 2.2.1). Several changes in rendering and feeding practices may have enabled the infectious agent to survive during rendering process and enter the cattle feed chain (Taylor, 1989, Horn et al., 2001). An alternative theory postulates a spontaneous case of BSE as the origin (Section 2.2.2). Section 2.3.1 discusses spontaneous disease in further detail. Additional theories focus on different infectious organisms or toxic agents that could cause a spongiform encephalopathy or on dietary imbalances known to produce spongiform encephalopathies under some conditions (Section 2.2.3).

2.2.1 Scrapie in Sheep

Evaluating the hypothesis that scrapie is responsible for the BSE epidemic in the UK is complicated by the fact that even transmission of this disease among sheep is not well understood. Horizontal transmission may involve the shedding of the agent into the environment (Hoinville, 1996, Stringer et al., 1998, Woolhouse et al., 1998). Maternal transmission from ewe to lamb in utero or immediately during the post-natal period is believed to occur, although there has been some debate in the past (Ridley and Baker, 1996). Maternal transmission of scrapie may

explain why the disease usually becomes endemic in a flock once it is introduced. Exposure through contaminated vaccines (Gordon, 1939, Gordon, 1946, Gordon, 1959, Agrimi et al., 1999, Caramelli et al., 2001) has been documented as a source of infection. Transmission of scrapie *via* vectors is disputed (Fitzsimmons and Pattison, 1968, Hourrigan et al., 1979).

The possibility that scrapie is responsible for the BSE epidemic in the UK is made more plausible by the fact that the size of the sheep population in the UK increased significantly from 1980 onwards. This growth may have lead to an increase in the prevalence of scrapie, a disease with an annual incidence now estimated to be between 5,000 and 10,000 per year in the UK (Hoinville et al., 1999). Moreover, it has been postulated that more scrapie-infected sheep than usual were introduced into the cattle feed supply during this period (Walker et al., 1991). In addition, changes in the rendering technology in the 1980s may have made this process less effective at deactivating the scrapie agent. During that time, meat and bone meal (MBM) was on the list of ingredients for "least cost" dairy calf starter rations and was regularly used as a source of alternative protein. If the species barrier can indeed be overcome by exposure to a sufficiently large amount of infectivity, and if young animals are especially susceptible to infection, these changes may have been sufficient to initiate the development of BSE in cattle. Once in cattle, according to this theory, the agent adapted, thus eliminating the species barrier, and quickly spread to other cattle through feed containing rendered ruminant material.

One finding supporting this theory is the observation that BSE apparently originated at several locations at nearly the same time (Wilesmith, 1994, Nathanson et al., 1997, Kimberlin and Wilesmith, 1994). Such a pattern suggests some sort of population-wide insult, such as a large supply of ineffectively treated feed containing scrapie. This theory is also supported by the finding that cattle are susceptible to infection by scrapie introduced by i.c. experimental inoculation. On the other hand, investigators have been unable to infect cattle with the North American scrapie agent when it has been orally introduced (Cutlip et al., 1994, Cutlip et al., 1997, Cutlip et al., 2001); research is ongoing using the UK scrapie agent (Linda Detwiler, personal communication). In addition, if scrapie did cause the development of BSE in cattle, it is not clear why it happened suddenly in 1986 at several locations given that scrapie has been endemic in European sheep for over 250 years and ovine and bovine wastes have been used in cattle feed for several decades. In response to that question, attention has focused on the use of MBM in dairy calf starter rations, feed given to very young animals (Horn et al., 2001). This practice, which is not likely to have taken place elsewhere (except in Australia, which is a scrapie-free country)

would have exposed animals to infectivity when they are most susceptible. Finally, if scrapie caused BSE to originate at several locations at nearly the same time, it is surprising that there is only a single strain of BSE even though sheep in the UK are known to carry several "strains" of scrapie.

One variation on the scrapie hypothesis suggests the existence of a strain of scrapie that was more thermostable and particularly infectious to cattle. This theory suggests that this strain may have entered the cattle feed chain as a component of MBM. The specific strain may have been a mutation of the scrapie agent. It is possible that either: 1) a single scrapie strain with characteristics unlike BSE was transmitted to cattle and that these characteristics changed as the agent was repeatedly recycled through cattle; or 2) a BSE strain pre-existed in sheep, and was unchanged when passed to cattle. These hypotheses are both plausible. However, if the origin of BSE was a single "strain" of scrapie, the BSE epidemic should have had a more geographically compact origin than the diffuse pattern actually observed, unless the single "strain" was widely distributed. BSE epidemiology shows a geographically widespread occurrence with simultaneous onset at multiple distant locations, rather than an origin focused at a single point. Finally, this alternative theory does not address the lack of a demonstration to date of *any* strain of scrapie that can infect cattle following oral administration (Unpublished, MAFF).

2.2.2 Infrequent Sporadic BSE

It is possible that BSE is a naturally occurring and long-established disease of cattle, but one that occurs extremely rarely (like sporadic CJD in humans). Passing infectious material from such an animal through a rendering process with greatly reduced capacity for destroying the agent could have led to contamination of the cattle feed chain. However, many countries had rendering systems similar to that of UK, so the absence of BSE in other countries, if it is indeed sporadic, seems unlikely. That is, we would expect to see native cases in other countries as well (*i.e.*, cases not traceable to UK). To date, none have been found (for a review see (Chesebro, 1999).

On the other hand, the cattle in the UK tend to be relatively old, with many dairy animals in particular of relatively advanced age. If sporadic BSE resembles sporadic CJD in humans, its incidence will be much greater in older animals. As a result, the UK herd may have been predisposed to an animal with sporadic disease approaching the highly infectious symptomatic stage of disease. The sporadic case theory postulates that a series of unfortunate events would

have had to coincide: 1) the rare sporadic case would have had to have been rendered; 2) the rendering would have had to leave enough of the infectivity intact to produce a sufficient number of "second generation cases"; 3) the rendered material would have had to have been used to produce feed for cattle; and 4) the repetition of this cycle. The apparent uniqueness of the UK as the origin of this disease may simply reflect better fortune in other countries.

2.2.3 Toxic Agents and Other Hypotheses

This section describes several alternative agents and conditions that have been suggested as possible causes of the BSE epidemic in the UK.

2.2.3.1 Organophosphate (OP) Pesticides

Organophosphate (OP) pesticide toxicity in cattle may resemble BSE, and like BSE, the clinical signs for OP toxicity exhibits seasonality. It has been suggested that there was a link between the use of some OP pesticides, especially Phosmet, and the development of BSE (Purdey, 1996). Adherents to this theory claim that the distribution and dynamics of the use of the pesticide are consistent with the epidemiology of the BSE epidemic in UK. However, the timing of the BSE epidemic's origin does not coincide with the extensive use of OPs in the early 1960's for warble fly control because most of the BSE cases were born after 1982. This hypothesis rejects the evidence that contaminated MBM plays a role in the transmission of the disease.

Purdey, the author of this theory, proposes that exposure of bovine embryos to high doses of Phosmet triggered the UK BSE epidemic (Purdey, 1996). The mechanism underlying this theory is the phosphorylation of PrP in fetuses of cows treated with Phosmet. The Spongiform Encephalopathy Advisory Committee (SEAC) concluded, however, that OP pesticides did not accumulate in cattle, which would be necessary for the transmission of the disease via contaminated feed. The committee agreed that the epidemiological evidence is more consistent with the hypothesis that the BSE epidemic was due to the widespread use of BSE-contaminated feedstuffs (SEAC, 1997). The EU Scientific Steering Committee (SSC) evaluated this hypothesis and determined that intoxication with OP compounds was consistent with some of the characteristics of the BSE epidemic but could not be considered to be the cause (SSC, 1998a). In particular, this theory fails to account for the presence of BSE cases in areas of UK that did not

use OPs (Horn et al., 2001), the absence of cases in areas of UK that did use OPs (Horn et al., 2001), and the absence of cases in countries that use OPs more extensively than the UK. In addition, OP exposure has not yet been shown to be transmissible.

2.2.3.2 Copper Deficiency

The incidence of BSE in the UK was highest in the southern and eastern counties of England (Wilesmith et al., 1991, Wilesmith et al., 1992). Several counties in this region are known to have widespread copper deficiencies in soils and crops (Thornton and Webb, 1979). These deficiencies could cause copper deficiency in ruminants. The resulting condition is known to have specific signs and pathological changes similar to those of BSE.

One theory for the origin of the BSE epidemic hypothesizes that the high levels of protein in feed used in the 1970s and 1980s competed with copper for absorption by ruminants (Rehbinder and Petersson, 1994). This theory is consistent with some aspects of the disease, but the morphology and distribution of vacuolar or spongiform-like changes observed in animals suffering from copper deficiency differ from the spongiform changes that are typical of a TSE (SSC, 2000a). This theory also fails to account for the disease's transmissibility. Furthermore, if this hypothesis were true, beef cows (that obtain most of their nutrients through pasture) should have a higher incidence of BSE than dairy cattle, and the reverse is observed (Horn et al., 2001).

2.2.3.3 Heavy Metal Exposure

The role of heavy metals in certain CNS diseases (Warren, 1974) has led some researchers to suggest they may have a role in the development of BSE. It has been shown that copper ions can convert PrP to the infective disease form (McKenzie et al., 1998). It has been suggested that contamination of MBM with heavy metals may have converted the normal PrP to the infective form. However, this theory is inconsistent with the characteristics of the epidemic in UK because heavy metal exposure is likely in areas where BSE has never been observed. Moreover, a potential source of heavy metal contamination of feed has never been identified, nor has transmissibility been demonstrated.