BOVINE SPONGIFORM ENCEPHALOPATHY – FOOD SAFETY IMPLICATIONS

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I. INTRODUCTION

"Absence of evidence is not the same as evidence of absence"
– M. J. Rees

Bovine spongiform encephalopathy (BSE) is a fatal brain disease of cattle presumed to have originated from the use of rendered scrapie-infected sheep offal as a dietary protein source for cattle. The oral route is the major route of natural transmission of BSE to cattle. Shortly after BSE was identified in cattle, a cluster of human cases of Creutzfeld-Jakob disease (CJD) with atypical characteristics occurred in the UK. This disease is referred to as "variant" CJD. Concern that BSE was spreading to man through the food supply engendered a flurry of research to ascertain whether, in fact, they were the same disease, and if so, whether they were spreading to man through the food supply.

II. BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

BSE was first observed in Great Britain in April 1985; it was specifically diagnosed in November 1986, when an affected cow was referred to the Central Veterinary Laboratory, Weybridge, England. By June 1990, there had been some 14,000 confirmed cases out of a cattle population estimated at 11,000,000 in the United Kingdom (UK). Since 1986, more than 170,000 cases of BSE have been identified in cattle in Britain. Over 34,000 herds have been affected; 64% have been dairy herds and 27% have been beef suckler herds (MAFF, 1998). BSE is predominantly a disease of dairy cattle; a total of 81% of all cases are of dairy origin (MAFF, 1998). The epidemic peaked in 1992–93 at almost 1000 cases per week.

When BSE was first observed in 1985, 200–300 infections had probably occurred already. Epidemiological data based on clinical onset (taking into account the long incubation period) suggests that most infections occur during the first two years of life (mean age at infection is 1.3 ± 1.8 years). The mean incubation period is five years (Anderson *et al.*, 1996a).

BSE has occurred predominantly in Friesian and Friesian crossbred cattle; while there are two genotypes of the bovine prion protein (PrP) gene, an analysis of 370 cattle in Scotland revealed no difference in the frequencies of the two prion genotypes between healthy cattle and those with BSE (Hunter *et al.*, 1989). Incidence is much higher in dairy herds and mixed herds (90% of cases), probably because cows are fed more protein and remain on feed for a longer period of time (Anderson *et al.*, 1996a, b).

In herds where supplementary feed is given to calves, feed intake rises with age for the first two years.

Other members of the *Bovidae* family are susceptible to BSE as evidenced by BSE (or very similar diseases) in captive nyala, eland, kudu, gemsbok, and oryx in Britain (Bradley, 1996). In addition to the 170,000+ cases in Great Britain, the disease has been reported in domestic cattle in the Republic of Ireland (337 cases), France (51), Portugal (195), Switzerland (283), Belgium (1), Luxumbourg (1), the Netherlands (2) and in cattle exported from England to Oman (2), the Falkland Islands (1), Germany (6), Denmark (1), Canada (1), and Italy (2) (MAFF, 1998). This number probably underestimates incidence since more than 2000 cases would be expected in the EU (outside of Britain) based on transmission epidemiology and exports of more than 70,000 metric tons of British meat and bone meal and 30–60,000 cattle between 1985 and 1990 (Anderson, 1996a; Blanchfield, 1996).

BSE is an afebrile, neurological disease; it elicits no immune or inflammatory response. Cattle affected by BSE experience a progressive degeneration of the nervous system. The onset of clinical signs is often associated with stress (herd change), calving, or estrus (Davis *et al.*, 1991). The average age of onset of clinical symptoms is 5 years, however BSE has been observed in cattle as young as 1 year 10 months; disease course varies from less than 2 weeks to 14 months but usually results in death or humane destruction within 4 months. Clinical signs include heightened sensory perception; changes in temperament, such as nervousness or aggression; abnormal posture, incoordination and difficulty in standing; excessive itching or licking; repetitive movements; decreased milk production; or loss of body weight despite continued appetite. The common name, "Mad Cow Disease", is related to abnormal motor nerve control coupled with aggressiveness, which are also symptoms of rabies.

Confirmation of disease is through post-mortem examination of brain tissue. Brain tissue sections of infected cattle appear spongy and infiltrated with amyloid (starch-like) plaques when examined microscopically (Davis *et al.*, 1991).

BSE is considered a "common source" epidemic; animals contract the disease from some common environmental element with which many come into contact. As such, the epidemic involves many individual, independent disease outbreaks. Epidemiological evidence indicates that the primary cause of BSE in British cattle was probably the use of commercial cattle feed concentrates which contained meat and bone meal derived from rendered sheep (and possibly cattle) presumed to have been infected with scrapie (Wilesmith *et al.*, 1991, 1992a, b). Current data are consistent with 90% of cases occurring from infected feed. The rapid growth of the

epidemic from 1986 to 1991 is consistent with recycling of contaminated material in the ruminant feed chain fuelling transmission prior to July 1988 (Anderson *et al.*, 1996a). In addition, there is a trend for the average age at clinical onset to decrease in cohorts born in 1977 compared to those born more recently, which is consistent with feed recycling fuelling the epidemic pace where a raised transmission intensity reduces average age at infection (Anderson and May 1991). The feeding of animal protein specifically derived from ruminants ceased in Great Britain in July 1988.

Agents which have been ruled out as the common source include semen, chemicals (organophosphate insecticides), biologics and pharmaceutical drugs. The use of meat and bone meal (MBM) in cattle rations as a protein source has been common for several decades. Changes in rendering operations in the 1970 and 1980s may have allowed transmission of the infective agents to cattle resulting in the large number of cases that developed (AABP 1996). The number of cattle in Great Britain has fallen steadily from 13.6 million in 1974 to 9.5 million in 1995. The estimated number of BSE infections in the UK over the period from 1974 through 1995 is 903,000; an estimated 729,000 entered the food chain (Anderson *et al.*, 1996a, b).

The oral route is the major route of natural transmission of BSE to cattle, which consume fairly small amounts of MBM relative to the number of infections which have occurred. Infection is dose-dependent but the required dose for BSE transmission to cattle is small; an oral dose of 500 mg to 1 g BSE-infected brain appears sufficient to be infective to calves (Anderson *et al.*, 1996a). Based on oral dosing using brain from BSE-infected cattle (1 g, 100 g, 300 g), incubation time appears to be dose-dependent also. Because of the build-up of infectious material in the latter half of the long incubation period, bovine tissues may reach an infectivity peak just when clinical signs of disease appear (Kimberlin and Walker 1988; Anderson *et al.*, 1996a; MAFF, 1997b).

Unlike scrapie in mice, where spleen and lymphatic tissues as well as brain are infectious, detectable titers of the BSE agent have been found only in brain, cervical spinal cord and dorsal root ganglia, retina, and distal ileum of cattle (Anderson *et al.*, 1996a). To date, BSE has not been experimentally transmitted by meat or milk. Taylor *et al.* (1995a) demonstrated that mice receiving milk from BSE-infected cows (orally, intracerebrally or intraperitoneally) showed no signs of neurological disease, spongiform encephalopathy or other specific pathology after two years.

Studies have been inconclusive with respect to vertical transmission (dam to fetus) in cattle. Placental transmission is a likely route for vertical transmission of scrapie in sheep (Ridley *et al.*, 1996) but has been inconclusive in cattle. A case control study to analyze the rate at which calves

born in close contact with BSE-infected cows at the time that they calved actually became BSE positive, found evidence of risk of transmitting infection horizontally up to three days after calving, but no evidence of transmission to the cow's own calf (Hoinville *et al.*, 1995). Calves fed with placenta from confirmed BSE cases were healthy for seven years (Hoinville *et al.*, 1995). The rate of maternal transmission appears to be approximately 10%, however under (UK) field conditions the maternal transmission rate has been estimated to be approximately 1% (MAFF, 1997b). A statistically significant correlation exists between BSE-infected dams and BSE occurrence in calves; however no causal relationship has been established (Anderson *et al.*, 1996a). Of the 903,000 cases that occurred in the UK between 1974 and 1995, an estimated 5100 arose through maternal transmission; an estimated 340 additional cases will occur by the year 2001. On July 20, 1996, the United Kingdom Spongiform Encephalopathy Advisory Committee (SEAC) stated that cows can transmit BSE to their calves, but the rate of transmission is so low that it is not expected to perpetuate the disease.

The BSE epidemic peaked in the winter of 1992/93 when approximately 1% of adult breeding cows were affected. The incidence has fallen by 40%/year since then and 50% in 1997. The within-herd incidence has been monitored as an indicator of whether or not horizontal transmission (cow-to-cow) is occurring. Within-herd incidence peaked in 1992 at 2.7% suggesting that there is little spread from animal to animal (Hoinville *et al.*, 1995; MAFF, 1997b).

Assuming the infectious agent is passaged via meat and bone meal in feed, the mechanism by which the infectious agent moves from the digestive tract to the central nervous system is mysterious. Spleen-derived white cells may carry the infectious agent through the lymph nodes; BSE infectivity has not been shown in the lymph nodes of BSE-infected cattle. However, traces of the BSE agent can be detected in the lymphatic tissues of the small intestines (Peyers patches) of calves receiving large doses of MBM from BSE-infected animals. Although some TSE-infected species have the TSE agent in their blood, this situation has not been demonstrated in BSE-infected cattle. If, however, the peripheral nervous system or the buffy coats of leucocytes provide the transmission route, it is possible that by the time the disease is apparent, the causative agent has left the peripheral system or is present at very low, undetectable levels (Almond, 1996). Taylor *et al.* (1996) have shown that scrapie can be transmitted to mice through cuts and abrasions on the skin which would indicate that blood or peripheral nerves transmit the infective agent to the CNS. The fact that BSE-infective agent is present in nerve tissue was the basis for the exclusion of all brain and spinal cord from meat and bone meal for animal feeds.

MAFF (1997a) reported the results of an ongoing experiment in which cattle were deliberately infected (high oral dose at 4 months) with BSE, developed clinical signs 35 months post-infection, and have been slaughtered at regular intervals as the disease develops. In addition to known tissues, infectivity has been detected in the dorsal root ganglia (DRG), trigeminal ganglia and, presumptively, in bone marrow. Trigeminal ganglia are in the skull (classified as SBM – see section XIII, "Control Measures in the UK"). DRG are swellings on the sensory branches of the nerves near the spinal cord. They are surrounded by the bone of the vertebral column. DRG infectivity was detected at 32 months but not at 26 months post-infection; infectivity has been detected 3 months but not 9 months before clinical symptoms developed. The implication is that an "incubating" animal has infective tissues but may have no clinical signs of disease.

In "created extremes", the species barrier between animals can be breached. Injection of infected material into the brain of another animal is a created extreme. BSE has been experimentally transmitted to a variety of other animals including rodents, cats and primates (MAFF, 1997b). Mice inoculated with BSE brain homogenates develop neurological signs of the disease 300 to 450 days later; this prolonged incubation period prior to onset reflects the species barrier between cattle and mice.

There are different scientific hypotheses concerning the origins of BSE. One theory is that BSE existed at very low and, therefore, undetectable levels in the British cattle population prior to 1988, as a rare sporadic disease, and that its increase in incidence was the result of numerous coincidental changes in the industry or environment. Experienced cattle practitioners claim to have seen BSE cases prior to the epidemic; these were (and in many countries, still are) often categorized as unresponsive "downer cows" or cows with "staggers". Whether or not these cattle acutally have or had BSE has not been substantiated. Another theory suggests that BSE initially appeared (as a new disease) in the 1980s and then spread unimpeded by the current industry practices and environment; no traces of a disease of this nature in cattle exists before 1985. This theory states that the endemic scrapie strains reached infective levels through recycling in feed. This subsequently resulted in some modification of the scrapie strain to the BSE strain. Alterations in rendering practices (primarily loss of solvent extraction of the fat prior to production of meat and bone meal) may have led to recycling (and concentrating) of the BSE infective agent through the cattle population via ruminant-to-ruminant feeding of rendered offal (as a protein source) until the titer was sufficient to result in an outbreak of sufficient magnitude that it was readily identified (Wood *et al.*, 1997). Another theory suggests that a new strain of scrapie, that was particularly infectious to cattle, arose; if this were the case, one would expect the new strain to

emerge first in a single flock of sheep resulting in a geographically localized epidemic which has not been the case (MAFF, 1997b).

A. CHANGES IN RENDERING WHICH MAY HAVE CONTRIBUTED TO BSE SPREAD

In the UK, the "fat melter" (knacker) removes dead animals (from farms, lairage, etc.), removes hides and hooves, and heats the remainder at a temperature (<80°C) just sufficient to separate the fat (which is sold). The remainder (greaves) is sold to the solvent extractors (Anderson *et al.*, 1996a). Inedible offal (heads, spleen, gut-associated lymphoid tissue, etc.) are rendered to produce tallow, and meat and bone meal. Meat and bone meal (MBM) was included in pig, poultry, pet and some dairy rations as a protein source. Inedible offal was processed either in batch or continuous systems; fat was removed after cooking by pressing, centrifugation or solvent extraction. Solvent extraction was used to increase the yield of tallow; however, sufficient heat (80°C for 8 hr followed by superheated steam) had to be applied to remove the residual solvent (Franco *et al.*, 1996). Rendering temperatures varied from 80–90°C in continuous low temperature wet-rendering systems to 140–160°C in high temperature dry-rendering systems (Morgan 1988). The UK methods for processing sheep carcasses changed in the 1970s (Taylor *et al.*, 1995b). Other countries were changing their rendering methods as early as 1960 (Jobling 1996). Prior to 1970, high temperature batch rendering was the predominant practice. In the mid-70s, rendering temperature was lowered. During the late 1970s and early 1980s, solvent extraction, typically applied to "greaves" after the primary cooking process, was abandoned on a large scale by the UK rendering industry (Taylor *et al.*, 1995b). Between 1978 and 1988, most offal renderers in the UK changed from batch to continuous systems (Morgan 1988). Processing temperatures were lowered to save energy and because solvent removal was no longer necessary (Morgan 1988). By 1982, nearly all rendering was without solvent at substantially reduced temperatures (AABP 1996). This may have allowed the infectious (scrapie and/or BSE) agent to concentrate (through recycling of infected animals) in MBM.

Apparently the pre-1970 processing methods eliminated the infective agent, or at least reduced the concentration, in the rendered materials prior to the inclusion of MBM as a supplement in cattle rations (Prusiner 1995). The cessation of solvent extraction coincides with the emergence of BSE in 1985 when the extended incubation time is considered (Wilesmith *et al.*, 1992b).

B. FEEDING PRACTICE CHANGES WHICH MAY HAVE CONTRIBUTED TO BSE SPREAD

Until scrapie was declared a reportable disease in the UK in 1993, infected sheep could be rendered. Up until 1993, UK animal protein in MBM contained at least 14% sheep tissue (versus 0.6% in the US prior to the voluntary ban). UK sheep slaughter is seasonal; during increased sheep slaughter periods, rendered protein may contain significantly more than 14% sheep material. The distribution of rendered protein from a single UK manufacturer tends to be national (wide scale exposure) (Morgan 1988), while it tends to be regional in the US (<150 miles from the processing plant) (AABP 1996). In the UK, rendered protein was often 4–5% of the total daily cattle ration; before the practice was banned, US cattle were fed <2.5% of total daily ration (Morgan 1988; AABP 1996). The use of ruminant MBM as feed for pre-weaned calves is quite common in the UK but much less so in the US (AABP 1996).

While rendering lambs ≤ one year old is estimated to result in a substantially reduced likelihood of scrapie transmission (because the agent has not yet reached the central nervous system) (Marsh 1990), renderers have difficulty determining the age of sheep, making the decision of which sheep to accept problematic. To reduce the problem, the approach has been to eliminate sheep from the rendering industry's raw materials supply.

III. PRIONS – THE DISEASE-CAUSING AGENT

BSE and other transmissible spongiform encephalopathies (TSEs) are not caused by a bacterium, virus or parasite; the causative agent contains no nucleic acid. These diseases appear to be caused by an unconventional infectious agent, originally described as a "slow virus," a "self-replicating protein" and more recently as a "prion" (proteinaceous infectious particle; Prusiner 1995). A prion is a normal protein (PrP) which can assume an abnormal (infective) conformation which is often referred to as PrP^{sc}, the scrapie isoform of the normal protein. The scrapie portion of this nomenclature refers to the fact that the most-studied TSE in animals is scrapie in sheep. The precursor protein, PrP, is a normal host-encoded glycoprotein present on/in nerve cell membranes; it has many O- and N-linked glycosylation sites in its precursor form (Brown *et al.*, 1990a). PrP is coded for by about 750 base pairs composing about 250 codons which code for the amino acids of PrP specific to the animal in question.

The gene for PrP is present in most mammals and in some other

warm-blooded animals (ratites), and is located on chromosome 20 in most. While the physiological function of PrP is uncertain, its conservation among mammals implies that it does/did have an important role (Oesch *et al.*, 1991; Roberts and James 1996). Normal PrP function has been evaluated by creating "knockout" mice in which the PrP gene has been inactivated by homologous recombination; these mice are resistant to prion diseases (Collinge *et al.*, 1995). As knockout (PrP null) mice age, they develop symptoms of neuronal disease, losing cerebellar Purkinje neurons and exhibiting weakened gamma-amino-butyric acid receptor mediated neuron activity (Sakaguchi *et al.*, 1995). The knockout mice have behavioral abnormalities like those in the human prion disease, Fatal Familial Insomnia (see section IV, "Related Prion Diseases"): altered circadian rhythms, fragmented sleep and sleep deprivation (Tobler *et al.*, 1996).

PrP and PrP^{sc} amino acid sequences (in an individual) are identical, meaning that PrP^{sc} is formed by some type of post-translational modification. The change appears to be in conformation or shape (Howard, 1996; Horwich and Weissman, 1997). PrP changes from predominantly alpha-helix to predominantly beta-sheet, creating PrP^{sc}. PrP is readily degraded by lysosomal proteases, while PrP^{sc} is protease-resistant. One or more PrP^{sc} molecules induce PrP molecules to change shape, probably by refolding, into PrP^{sc}. Normal cellular PrP can be converted into PrP^{sc} *in vitro* by simply mixing the two proteins together (Lansbury and Caughey, 1996). It has been speculated that heritable disease variability associated with different "strains" of the infectious agent are caused by the self-propagation of PrP^{sc} polymers with distinct conformations; Lansbury and Caughey (1996) have proposed a nucleated-polymerization model of PrP^{sc} replication. In any event, the infectivity ratio of PrP^{sc} (ability to induce conversion of the normal to the infective form) compared to PrP (ability to spontaneously convert to the infective form) is on the order of 100,000:1 (Heaphy, 1996).

What triggers the original conformational change of normal PrP to aberrant (disease-causing) PrP^{sc}? While the spontaneous conversion of PrP to PrP^{sc} seems to be thermodynamically unfavorable (corresponding to a low incidence of sporadic transmissible spongiform encephalopathies such as CJD, and probably responsible for the long incubation time even in those individuals with mutated PrP genes), mutations in the PrP gene may make the spontaneous conversion more likely (Kacser and Small, 1996). When the tendency to "spontaneously" convert from the PrP to the PrP^{sc} form is due to a genetic mutation in the original PrP, the mutation is usually due to a substitution/insertion of incorrect amino acids at those positions that would destabilize a helix increasing the likelihood that the affected helix

(and its neighbors) will refold into a beta-sheet form (Cohen *et al.*, 1994). There are at least 20 known mutations in the PrP gene sequence resulting in "spontaneous" PrP^{sc} formation. Each mutation results in a slightly different conformation, and slightly different pathology. Regardless of the mutation, the "spontaneously formed" PrP^{sc} can go on to change non-mutated PrP into PrP^{sc}; this conversion may be much more thermodynamically favored than the original conversion (Heaphy, 1996). The process is self-perpetuating.

Peripheral introduction (ingested or inoculated) of PrP^{sc} can also convert normal PrP to PrP^{sc}. At some point, infectious PrP^{sc} gains access to the nervous system where it may travel from peripheral sites (wounds) or internal organs (spleen) to the central nervous system (Kimberlin, 1990). In sheep, PrP^{sc} appears to be transported and may replicate in the lymphoid systems (Peyers patches, follicular dendritic cells). The host-cell involved in BSE (and some other TSEs) prion uptake and transport to the nervous system is not known (Bradbury, 1997).

PrP is anchored to the outer surface and extends through the cell membrane of neurons (in most tissues and species); it occurs to a lesser extent on lymphocytes and some other tissues (Bueler *et al.*, 1992). Blatter *et al.* (1997) reported experimental evidence that PrP-expressing tissue is required for the transfer of scrapie infectivity from spleen to brain; expression of PrP in a tissue compartment cannot be reconstituted by bone marrow transfer, therefore the requirement for the normal isoform of PrP in peripheral tissues represents a bottleneck for the spread of prions from peripheral sites to the central nervous system. Perini (1996) reported that platelets express normal PrP (bound to the cell membrane by a GPI-anchor); they shed this protein upon agonist stimulation. Because platelets express PrP at the surface, it is possible that they could be converted to PrP^{sc} then transported throughout the body. The CJD infective agent has been found in buffy coat preparations at various times during the preclinical phase of the disease as well as during the clinical phase (Manuelidis *et al.*, 1978). Animals infected intracerebrally with PrP^{sc} exhibit infectivity in the spleen (the major site of platelet catabolism) two weeks after injection.

Once in the neurons, PrP^{sc} autocatalytically converts normal PrP to PrP^{sc} (Heaphy, 1996). The nerve cell eventually attempts to degrade the prion in the lysosome, however PrP^{sc} protein is very resistant to endogenous proteases that would normally break down the protein. It may be partially cleaved, producing PrP^{sc} fragments which build up, fill the lysosome then kill the cell (leaving holes known as "spongiform" damage). These fragments aggregate and precipitate forming plaques. However, even though significant amounts of tissue damage are occurring, white blood cells do

not infiltrate the brain indicating that no inflammatory response is generated.

For the same reasons that cells cannot destroy PrP^{sc}, prions are very resistant to a variety of denaturants (see section X, "Inactivation of Infective Agents").

IV. RELATED PRION DISEASES

BSE belongs to a group of related diseases known as the transmissible spongiform encephalopathies (TSEs) which have very similar characteristics. Clinical signs are primarily neurological: behavior changes, repetitive movements, difficulty walking/balancing, and muscle spasms, as well as wasting in spite of food intake. All cause fatal spongiform changes in the brain; some cause amyloid plaque development. None cause inflammation.

The incubation period of these diseases tends to be quite long: from months (rodents, cats), or years (cattle, goats, sheep, captive ungulates) to decades (man). The extended incubation period results in clinical signs appearing at maturity, often followed by long, progressive neurological compromise. Detectable titers of abnormal prion change during the prodromal phase; they seem to rise exponentially during the second half of the incubation period in the brain but may plateau earlier in spleen and lymph tissues, and then decline (prior to clinical symptoms) (Kimberlin and Walker, 1988; Anderson *et al.*, 1996a).

This group of diseases includes scrapie, which affects sheep and goats; chronic wasting disease (CWD) of mule deer and Rocky Mountain elk; transmissible mink encephalopathy (TME); feline spongiform encephalopathy (FSE); and four rare human diseases – kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), and Fatal Familial Insomnia (FFI). Spongiform encephalopathies (similar to BSE) have been reported in the UK in other members of the *Bovidae* family (6 kudus, 4 eland, 1 nyala, 1 gemsbok and 2 oryx) and the *Felidae* family (100 pet cats, 4 cheetah and 1 puma) (Kirkwood and Cunningham, 1994), and in the *Cervidae* (Rocky mountain elk, deer) and *Mustelidae* (mink) families in the US.

A. SCRAPIE

Scrapie is a progressive, fatal neurological ovine (sheep) and caprine (goat) disease which was first reported in 1732. Scrapie is so-named because of the pruritus-induced rubbing and scratching (scraping on fences, etc.)

resulting in loss of wool. When scrapie is transferred into rodents, the animals lick and groom excessively. Scrapie is the "archetypical" spongiform encephalopathy (Groschup and Haas, 1996). It appears to be the only "naturally" occurring spongiform encephalopathy (SE) that can be transmitted both horizontally (animal-to-animal) and vertically (dam-to-fetus). All other animal spongiform encephalopathies appear to be artificially transmitted with the infected animals becoming "dead-end hosts" (Schreuder, 1994). Recent, but scanty, data on CWD in captive and free-ranging ungulates (*Cervidae* and *Bovidae*) may ultimately show that SEs do exist naturally in other animal species.

Scrapie in sheep has been endemic in all countries except Australia and New Zealand for 200 years (Kimberlin, 1981). One third of all British flocks have had at least one case of scrapie. Overall scrapie prevalence in the UK appears to be between 0.5 and 1.0% (Morgan *et al.*, 1990). It is estimated that about 1% of US sheep have scrapie; scrapie is a reportable disease in the US. Some 90–95% of cases occur in the Suffolk or Hampshire breeds. Typically, no more than 5% of the animals in a herd are infected. The US has had a USDA APHIS voluntary scrapie-free flock certification program since October, 1992.

Scrapie infection results in widespread neuronal degeneration, characterized by shrinkage, and vacuolation and hyperchromia of the cell body. These changes ultimately result in the deposition of "Scrapie Associated Fibrils" (SAF) as plaques in and around nerve cells. As early as 1959, Hadlow commented on the similarities in the histopathologies of scrapie and kuru in man. The SAFs in scrapie plaques are composed of sulfated glycosaminoglycans for which immunoreactive detection methods have been developed (Guiroy *et al.*, 1991). Presence of immunoreactive SAF occur in many other animal SEs.

Scrapie is an acquired infection with an incubation period of 2–5 years (Schreuder, 1994). At least 20 "strains" of scrapie with different incubation times and histopathological lesion profiles have been identified (Bruce and Fraser, 1991; Bruce *et al.*, 1994). It can be naturally transmitted between sheep through the placenta or by contact with the placenta. With respect to vertical transmission (dam to fetus), Foster *et al.* (1992) reported that when embryos were transferred from scrapie-infected sheep to non-infected sheep, 6 out of 26 (23%) dams appeared to have transferred the disease to their young *in utero*. More recently, *in-utero* vertical transmission (dam-to-offspring) has been disputed (Ridley and Baker, 1996). Parturient ewes are also responsible for spread of infection to unrelated animals via placental tissues, at least under experimental conditions (Pattison *et al.*, 1972). Because the average age of scrapie onset is 2.5 years (Gustafson, 1991) rendering of lambs less than

one year of age is thought to eliminate 90–99% of the infectivity (Brown *et al.*, 1982).

The scrapie agent can be recovered from lymphatic tissue, tonsils, spleen and thymus as well as nervous tissue. Infection can originate from pasture where placental tissue carrying the agent is ingested. Scrapie has been experimentally transmitted (intracerebrally) to a number of animals including rodents (hamsters, mice) mink, ferrets, goats, monkeys and cattle. Oral infection has been successful in mice (Chandler, 1963), sheep and goats (Pattison *et al.*, 1972), and squirrel monkeys (Gibbs *et al.*, 1980).

The scrapie agent is highly resistant to a variety of extreme conditions and may build up over time. Brown and Gajdusek (1991) reported that infectious material buried in the soil lost only about 50% of its infectivity in three years. Eradication attempts in Iceland failed when scrapie-free sheep were restocked onto pastures that had been kept free of sheep for three years (Sigurdarson, 1991).

It appears unlikely or at least extremely unusual for scrapie to be transmitted to man either occupationally or by eating lamb or mutton. While scrapie is common in sheep, CJD – the most similar human TSE – is very rare in man.

B. CHRONIC WASTING DISEASE (CWD) OF MULE DEER AND ROCKY MOUNTAIN ELK

Chronic wasting disease (CWD) is a progressive neurological disorder that affects captive mule deer, black-tailed deer, hybrids of mule and white-tailed deer, and Rocky Mountain elk in the US. CWD was first observed in captive mule deer in a wildlife facility in Colorado in 1967 (Williams and Young, 1980). Approximately 90% of the affected mule deer were hand-raised from infancy in the facility. The disease has a high morbidity in young adult animals which have been maintained in captivity for periods of 2.5 to 4 years. The majority of animals suffer progressive weight loss and depression over 2 weeks to 8 months leading to emaciation and death. Most deer have signs of polydipsia, polyuria, excessive salivation, grinding of the teeth, lowering of the head, drooping of the ears and terminal anorexia. CWD appeared in Rocky Mountain elk in 1979 in the same wildlife facility. Elk had sporadic fence-line contact with affected deer, as well as with other free-ranging species and were occasionally maintained in pens that had previously held CWD-affected deer. Clinical signs occurred in elk after 3–5 years in captivity; the clinical course of the disease was 1–6 months and was characterized by nervousness, progressive weight loss, excessive salivation, teeth-grinding, lowering of the head and ears, but polydipsia, polyuria and pruritus were not observed (Williams and Young, 1982).

Williams and Young (1992) reported animal-to-animal (horizontal) transmission of CWD in captive cervids. CWD is transmissible to healthy mule deer (and ferrets) by intracranial inoculation of brain suspension resulting in a 17–21-month incubation period (Williams and Young 1982).

Histopathological examination shows amyloid plaques in central nervous system tissues of >60% of spontaneously affected animals between 2 and 4 years of age (Bahmanyar *et al.*, 1985). The amyloid plaques are reactive to antibodies directed against scrapie amyloid plaque (protease-resistant protein 27–30) in both elk and deer (Guiroy *et al.*, 1991). Consistent, severe lesions occur in the olfactory tubercle and cortex, hypothalamus, and parasympathetic vagal nucleus; the duration of clinical disease is unrelated to lesion distribution or severity (Williams and Young, 1993).

Between 1981 and 1995, a spongiform encephalopathy was diagnosed in 49 free-ranging cervids from northcentral Colorado in the general vicinity of the facilities where captive cervids with CWD are housed. Mule deer were the primary species affected accounting for 84% of the cases; the remainder were Rocky Mountain elk. Cases increased from <one/year to >one/month over the period. Scrapie-associated prion protein was demonstrated (antigenically) in brains from 26 animals. Clinical signs, gross and microscopic lesions were indistinguishable from those in CWD in captive cervids. Spraker *et al.* (1997) believe these cases to be naturally occurring SE caused by an agent identical to that of CWD.

Currently, CWD appears to be endemic in the facilities (Colorado and Wyoming) where it was first reported. Two eradication attempts have failed; one involved extensive disinfection of structure and pastures and disposal of all animals. The area was kept free of *Cervidae* for one year then restocked with captured wild elk calves. Some cases of SE have occurred since (Williams and Young, 1992). The source of SE in free-ranging animals is undetermined.

Reports on CWD of captive mule deer and SE in elk in the US drew little attention until the BSE outbreak in the UK where SE in other captive ungulates (nyala, eland, gemsbok, kudu and oryx) have occurred. All UK cases occurred in members of the order *Bovidae* with no cases in the order *Cervidae* while the reverse appears to be true in the US (among captive ungulates).

C. FELINE SPONGIFORM ENCEPHALOPATHY (FSE)

The first case of feline spongiform encephalopathy (FSE) in a domestic cat was reported in 1990 by Wyatt *et al.* By 1996, 100 cases had been reported in British cats (Corbin, 1996). FSE in captive *Felidae* has occurred in

cheetahs and puma (Kirkwood and Cunningham, 1994). All cats demonstrate progressive, neurological disease involving locomotor disturbances and abnormal behavior. The histopathological changes in the central nervous system are indistinguishable from scrapie-like encephalopathies (Schreuder, 1994). Scrapie-associated fibrils occur in brain extracts as does PrP^{sc} (Pearson *et al.*, 1992). Although many of the symptoms are the same, this disease appears to be new and distinctly different from "feline dysautonia" which does not include the typical spongiform lesions (Schreuder, 1994).

The source of FSE is not known but has been presumed to be pet food contaminated with the same material that caused BSE in the UK: scrapie-infected sheep and goat meat plus bone meal products (Schreuder, 1994; Corbin, 1996). This practice was banned in the UK in 1988 (Coles, 1991).

All of the large carnivores affected were fed parts of cattle carcasses judged unfit for human consumption, usually from the knacker yards (Kirkwood and Cunningham, 1994). Generally, the animal's diets consisted of meat and bone but occasionally included heads and split spinal columns. When the disease was passaged into mice, the symptoms and "lesion profiles" matched those of mice into which BSE from cattle had been passaged (Bruce *et al.*, 1997).

D. TRANSMISSIBLE MINK ENCEPHALOPATHY (TME)

Transmissible mink encephalopathy was first reported in 1947 in Wisconsin (Hartsough and Burger, 1965). TME is a relatively rare disease in ranch-raised mink, occurring only sporadically; some 23 outbreaks have occurred worldwide (USA, Canada, Germany, Finland, and the former USSR) since its identification (Marsh and Hadlow, 1992). Mink, members of the *Mustelidae* (marten) family are carnivores, like the *Felidae*. Sporadic cases have been attributed to the feeding of scrapie-infected sheep offal and carcasses, however direct (experimental) transmission via the oral route has not been demonstrated (Marsh *et al.*, 1991; Marsh and Bessen, 1993). Scrapie-infected materials as the source of TME has been questioned since the 1985 TME outbreak in Stetsonville, Wisconsin, where ranch mink were fed only "downer" or dead cattle (not sheep). The incubation time in inoculated mink is about 7 months (Marsh, 1991). The spongiform changes occurring in TME can be much more severe than the degeneration occurring in some breeds of scrapie-infected sheep and goats (Marsh *et al.*, 1976). Aged mink (18 months old) that are homozygous for the Aleutian (gunmetal coloring) gene exhibit much less severe changes (Marsh *et al.*, 1976).

Marsh *et al.* (1991) inoculated (i.c.) Holstein bull calves with mink brain material causing fatal SE in the cattle within 19 months. The SE in the cattle was different from typical BSE. Infectious material from the cattle was inoculated (i.c. and p.o.) back into healthy mink resulting in a similar SE to TME with short incubation time. The agent was transmissible to Syrian hamsters and ferrets from either mink or cattle but not to mice. Marsh and Bessen (1993) state that these results suggest the presence of an unrecognized BSE-like disease in the US. Mink ranchers often feed their mink animals that died of unknown causes, road-kill, offal, and 4-D animal products.

E. HUMAN SPONGIFORM ENCEPHALOPATHIES

Human prion diseases share a number of well-recognized characteristics such as long incubation times prior to clinical symptom followed by rapidly progressing dementia, myclonus (seizure involving spasms of individual muscles) and widespread spongiform degeneration (Richardson and Masters, 1995). The neuropathology of the human TSEs is characteristic: neuronal cell death, spongiform changes, gliosis, astrocytosis but no inflammatory responses. Amyloid plaques containing PrP are nearly always observed in many brain regions (Prusiner, 1993).

Human prion diseases can be (1) acquired by ingestion (kuru: transmitted by cannibalistic funeral rituals), (2) acquired by an iatrogenic route (surgery, cadaveric growth hormone injection, corneal transplant), (3) hereditary transmission as an autosomal, dominant trait, and (4) sporadic – of unknown origin (Howard, 1996). About 15% of human prion diseases are inherited. All families identified to date with inherited forms of these diseases have coding mutations in the PrP gene which may be either point mutations or insertions (Collinge and Palmer, 1994; Prusiner and DeArmond, 1994; Richardson and Masters, 1995). There are at least 80 positions in the full open reading frame of the gene for PrP protein that could lead to a minimum of one amino acid replacement; this leads to 3160 possible different pairs of amino acid replacements (Goldman *et al.*, 1996). Families with the genetic mutations commonly lose half their members to prion diseases. The majority (80–85%) of human prion disease is sporadic; these may arise from unidentified somatic mutations of the PrP gene, spontaneous conversion of PrP to PrP^{sc}, or as a result of unidentified exposure to an environmental source of either human or animal prions (Howard, 1996). Palmer *et al.* (1991) reported that 21 of 22 sporadic CJD cases (and 19 of 23 suspected cases) were homozygous at polymorphic amino acid residue 129.

1. Kuru

Kuru, first reported in 1900, was intensively studied in the 1950s and 1960s after it was identified in isolated tribes in the Fore highlands of New Guinea by Vincent Zigas and Carleton Gajdusek (Prusiner, 1995). Many highlanders became afflicted with a strange, fatal disease which they called "laughing death," because of the facial grimace (smile) which the neurological damage causes. The disease characteristics include the shortest incubation period of any human prion disease (4 to 5 years; no species barrier exists), cerebellar ataxia, inability to maintain posture, behavioral changes, late-stage dementia and myoclonus. The cause was determined to be ritual cannibalism of the brains of the dead; the tribes believed this practice afforded the living the knowledge obtained during the life of the deceased. Brain tissue was ground into a pale grey soup, heated and eaten. This ritual was practiced primarily by adult women and children of both sexes; men consumed muscle and other tissues. Since ritual cannibalism stopped, kuru has virtually disappeared.

The similarity between kuru and scrapie is striking (Hadlow, 1959). The large single or multilocular "soap bubble" vacuoles in the cytoplasm of nerve cells have long been regarded as a characteristic finding in scrapie; this unusual change is seldom seen in human neuropathological disease but is found in kuru (Hadlow, 1959). In 1966, Gajdusek *et al.* were able to experimentally transmit (via intracerebral inoculation) a kuru-like syndrome from infected animals to chimpanzees. Since 1957, 2600 kuru cases have been identified (Prusiner, 1995).

2. Creutzfeldt-Jakob disease (CJD)

CJD, first described in 1920, is the most common clinicopathological subtype of human transmissible spongiform encephalopathy. It occurs in inherited, acquired and sporadic forms. The inherited forms (including familial CJD) are autosomal dominant disorders associated with coding mutations in the PrP gene. The majority of cases occur as a randomly distributed illness of unknown cause (sporadic CJD) or as an acquired disease transmitted from an infected host (Prusiner, 1993). In the 1960s, CJD was shown to be transmissible in the laboratory. Iatrogenic CJD, resulting from use of cadaveric growth hormones and transplant tissues, or contaminated instruments, has been recognized for the last 30 years, but is relatively rare.

Classically, sporadic CJD occurs in those >65 years of age and presents as rapidly progressive dementia with myoclonus, characteristic EEG (triphasic spike signal at 1 cycle/sec), progressing via loss of movement

sensation (akinetic mutism) to death usually following hypostatic pneumonia in less than 6 months (Roos *et al.*, 1973; Brown *et al.*, 1986a; Collinge *et al.*, 1990).

Some CJD cases are associated with various mutations or insertions in the open reading frame of the prion protein gene (Pickering-Brown *et al.*, 1995). Some 8 polymorphisms in the PrP gene have been associated with familial CJD in some 100 different families; a total of 13 recognized mutations (at codons 102, 105, 117, 129, 145, 178, 180, 198, 200, 210, 217, 232 and insertions) are associated with the inheritied human prion diseases (Palmer and Collinge, 1993; Prusiner, 1995). In contrast to scrapie in sheep, familial CJD is inherited in an autosomal dominant fashion (Collinge *et al.*, 1995).

A common protein polymorphism of human PrP, methionine or valine homozygosity at residue 129, is often predictive of CJD susceptibility (Owen *et al.*, 1990; Palmer *et al.*, 1991). The distribution of these polymorphisms in the Caucasian population are (Owen *et al.*, 1990): 11% v/v, 38% m/m, 51% m/v. The distribution of these polymorphisms in individuals who have sporadic CJD risk is: 69% v/v, 25% m/m, 6% m/v. The distribution of these polymorphisms in individuals who have iatrogenic CJD from human cadaver growth hormone is: 50% v/v, 31% m/m, 19% m/v (Collinge *et al.*, 1991; Brown *et al.*, 1994b). The new variant CJD (vCJD) patients have all been m/m genotype (Palmer and Collinge, 1993). Heterozygosity at codon 129 protects individuals from CJD. This supports the current model of prion propagation wherein some PrP^{sc} molecules act as templates to promote conversion of PrP to the infectious isoform, a process which would be most efficient when the interacting species have identical primary structures (Prusiner *et al.*, 1990; Howard, 1996).

Pickering-Brown *et al.* (1995) reported that, of 20 cases of sporadic CJD in the UK, those homozygous for methionine at codon 129 had no amyloid plaque development while those homozygous for valine at codon 129 had extensive plaques (clinically resembling Fatal Familial Insomnia). This same group reported that the mean age of onset of sporadic CJD was affected by the patient's apolipoprotein E (ApoE) variant: Apo E2 – 67 years; Apo E3 – 60.1 years; Apo E4 – 63.5 years. Apo E has been shown to bind to amyloid plaques (Nakagawa *et al.*, 1995). Pickering-Brown *et al.* (1995) suggest that the prion could enter the cell through the Apo E/LDL receptor route. The Apo E2 isoform binds the LDL receptor with much reduced affinity compared to E3 and E4 which would result in reduced prion uptake slowing down the disease process.

The world-wide incidence of (sporadic) CJD is 1/1,000,000 population (at any one point in time). This translates into a lifetime risk of 1/10,000 (Heaphy, 1996). In the US, CJD has remained relatively

constant at about 1/1,000,000 (Holman *et al.*, 1995). The National Center for Health Statistics (CDC, 1996) reports the annual CJD mortality rates in the US 0.8/million in 1979, 1.1/million in 1987, and 0.8/million in 1992.

As an iatrogenically acquired disease, CJD can be transmitted by exposure of healthy recipients to tissues from infected donors; tissues known to transmit the disease include dura mater grafts, retinal transplants, and pituitary-derived hormones (growth, gonadotropins) either through intentional exposure or through use of contaminated surgical equipment (Flanagan and Barbara, 1996). The question of transmission via peripheral routes (oral, peripheral injection) remains open. Prion protein has been found in the blood of CJD-infected humans (Manuelidis *et al.*, 1978, 1985), however it appears that the concentration is so low that it is undetectable in many studies of TSE transmission (Dealer, 1996). Whether the concentration is sufficient to transmit the disease is unknown.

3. New Variant CJD (vCJD)

In 1995, Britton *et al.* and Bateman *et al.* identified two cases of CJD in teenagers that had unusual kuru-type plaques. These plaques are normally seen in only 5% of CJD cases. These patients had no recognized risk factors for CJD (iatrogenic routes or previously recognized prion gene mutations). However, they did have some familial history of dementia (Collinge *et al.*, 1996). Consideration of the medical histories, genetic analysis, possibility of increased ascertainment and other possible explanations failed to provide an adequate explanation for the pattern.

Subsequently, a consistent and previously unrecognized disease pattern was identified in 10 (currently 34) other cases of CJD (Will *et al.*, 1996; MAFF, 1998). Some of the features of the disease are different from those of classic CJD. Patients have behavioral and psychiatric disturbances (depression, personality change), early and progressive ataxia (failure of muscular coordination), memory impairment, dysesthesia and foot pain. The new cases had extensive plaque formation and identifiable patterns of prion protein immunostaining. These cases were similar to iatrogenic CJD associated with peripheral inoculation of prions (cadaveric growth-hormone cases) and kuru which are also characterized by progressive ataxia, behavioral and psychiatric disturbances. This cluster is referred to as variant-CJD (vCJD) (Will *et al.*, 1996).

These cases occurred in people under 42 years of age and were histologically distinct from classical CJD. Among 185 cases of sporadic CJD identified in the UK between May 1990 and April 1996, the aver-

age age at onset was 65 years and median duration was 4 months (Will *et al.*, 1996); vCJD cases averaged 27.5 years of age at onset and a median duration of 13 months. vCJD cases exhibited atypical EEG recordings. The key histological feature was the presence of multicentric, florid, amyloid plaques (resembling kuru) with eosinophilic centers and pale peripheries surrounded by spongiform lesions (similar to scrapie) in brain sections examined postmortem. Plaques were extensively distributed throughout the cerebrum and cerebellum; spongiform changes were most evident in the basal ganglia and thalamus. All vCJD patients for which genotype was available were methionine homozygotes at codon 129 of the PrP gene. Using established diagnostic criteria for CJD, none of these cases would have been classified as "probable" cases of CJD on clinical grounds (Will *et al.*, 1996). The unique aspects of vCJD allowed for a case definition to be established enabling future surveillance.

On 21 March 1996, Dr R. G. Will of the (UK) National Creutzfeldt-Jakob Disease Surveillance Unit asked neurologists to notify the CJD Surveillance Unit of any cases, past or present, with the following clinical or neuropathological profile: an early age of onset or death; prolonged duration of illness; predominantly psychiatric presentation including anxiety, depression, withdrawal and behavioral change which progresses; presentation with dysesthesia in the limbs and/or face; development of a cerebellar syndrome with gait and limb ataxia after a period of weeks or months; forgetfulness/memory disturbance developing, often late in the clinical course but progressing to severe cognitive impairment and a state of akinetic mutism; myoclonus sometimes preceded by choreiform movements, but typical EEG of CJD is absent; neuropathological spongiform changes including neuronal loss and astrocytic gliosis most evident in the basal ganglia and thalamus; and, (most striking and consistent neuropathologically) amyloid plaque formation extensively distributed throughout the cerebrum and cerebellum. A similar World Health Organization (WHO) case definition of the new CJD variant (vCJD) (Roberts and James, 1996) soon followed.

4. Gerstmann-Straussier-Scheinker Syndrome

GSS was described in 1936. In the early 1960s, GSS was known to be familial; the gene was not described but it was known to be autosomal and dominant. Some 50 extended families have been identified as having GSS (Prusiner, 1995).

The incidence of GSS is about 2% that of the rate of CJD (Heaphy, 1996). GSS is differentiated from CJD in that it presents in the 40s or 50s,

and is characterized by cerebellar ataxia and motor problems. Dementia is less common than with CJD. The disease course is several years. GSS occurs in both familial and sporadic forms. The familial form often occurs due to a mutation at codon 102 where leucine is substituted for proline in the PrP (Prusiner, 1995). Two unrelated families have the same double mutation: codon 178 (Asp/Asn) and codon 200 (Glu/Lys). Goldfarb *et al.* (1992) reported the presence of an insert mutation in the chromosome 20 amyloid precursor gene in a GSS family.

GSS diagnosis is confirmed by histopathological examination of the brain postmortem. Characteristics include non-inflammatory lesions, vacuoles, amyloid protein deposits, and astrogliosis.

5. Fatal Familial Insomnia (FFI)

FFI was described in 1986 as a disease beginning in adult life which follows a short but fatal course, and is characterized by severe selective atrophy of the thalamus (Lugaresi *et al.*, 1986). FFI susceptibility is passed through autosomal dominant inheritance. The age of onset of disease varies between 37 and 61 years and disease course averages 13 months. Progressive insomnia, autonomic disturbances (hyperhydrosis, hyperthermia, tachycardia, hypertension) and motor abnormalities (ataxia, myoclonus) are always present but in varying degrees. Insomnia with hallucinations and vigilance disturbances progress into stupor and coma. Hormonal disturbances (ACTH, cortisol, prolactin and melatonin) may be responsible for abnormal circadian rhythms. The insomnia appears to correlate well with thalamic pathology (Manetto *et al.*, 1992). The genetic mutation most commonly associated with FFI is at codon 178 of the PrP gene: the substitution of aspartic acid by asparagine (GAC to AAC) (Medori *et al.*, 1992). Nine extended families have been identified with genetic mutations associated with FFI (Prusiner, 1995).

V. GENETICS OF PRION DISEASES

The appearance of TSEs in specific subgroups of populations (breeds of sheep, cattle, mink, and human families) has led to the suspicion that TSEs have some genetic component. Normal cellular prion protein, PrP, is encoded by a single exon of a single copy gene – the PrP gene – which is found on chromosome 20 (in humans). PrP is found predominantly on the neuronal cell surface attached by a glycoinositol phospholipid anchor that passes through the membrane.

Studies of scrapie in naturally infected sheep show that the genetic

background of the host plays a major role in the disease course. Sheep challenged with a common mixture of infectious brain material vary in susceptibility. Dorset Downs are "fully resistant" (0/48 animals affected in 24 months); Herdwick are "highly susceptible" (28/36 died) (Gordon, cited in Prusiner, 1984). Survival time of sheep is thought to be determined by or in the region of the *Sip* gene (Scrapie Incubation Period), a single locus gene with two alleles (sA = short and pA = prolonged) that have some bearing on scrapie incubation time in that genotype of sheep (Dickinson, 1976). Sheep homozygous for the sA allele are susceptible; heterozygotes are susceptible but have a longer incubation period; pA homozygotes are resistant depending on infection route (Kimberlin, 1990). Natural scrapie occurs primarily in the sAsA genotype and is rare in the sApA genotype (Schreuder, 1993). Scrapie-susceptible sheep have been shown to be homozygous for the PrP^{VQ} allele (Val at position 136, Glu at 171); more resistant sheep seem to be heterozygous possessing one PrP^{VQ} allele and one PrP^{AR} (Ala at position 136, Arg at 171). This form of heterozygosity has been shown to confer scrapie resistance in several breeds (Belt *et al.*, 1995; Schreuder *et al.*, 1996). A similar situation exists in mice; the *Sinc* gene (Scrapie INCubation period) also has a short (s7) and prolonged (p7) allele. Passage of scrapie through various mouse genotypes will result in different incubation periods (an important aspect of SE strain typing). Most scrapie strains give a short incubation period when sub-passaged in $Sinc^{s7s7}$ mice and a long incubation period when sub-passaged in $Sinc^{p7p7}$ mice (Dickinson and Meikle, 1971).

When amino acid-identical, same-source PrP^{sc} is injected into animals with different genetics, the disease expression may be radically different. The paradigm in classical genetics is "one genotype, one phenotype". However, there are "strains" of scrapie that do not exhibit the usual incubation time pattern when sheep of known genetics are experimentally infected. Most strains have incubations which are predictable based on the *Sip* gene predictions (e.g. SSBP/1 strain, Hunter *et al.*, 1989), however there are strains (e.g. CH1641, Foster and Dickinson, 1988) that produce the opposite effects. These facts may be somewhat resolved by considering the internal make-up of the PrP gene. The PrP gene contains a number of internal polymorphisms which result in substitution of one amino acid for another at a specific region. In the murine gene, polymorphisms often correspond with differences in incubation time (Westaway *et al.*, 1987). The *Sip*-connected polymorphisms seem to be closely linked with the PrP gene but do not necessarily reflect changes *within* the PrP gene (Schreuder, 1994). Goldmann *et al.* (1991) identified three polymorphisms inside the coding region of the sheep PrP gene in codons 136, 154 and 171 which resulted in PrPs with different amino acids at three positions: 136 Ala/Val,

154 Arg/His and 171 Arg/Gln. The substitution of Val for Ala at 136 was linked to short incubation period (sA allele of *Sip* gene). Homozygosity for 136/Val has been found only in scrapie-positive Cheviot, Ile-de-France and Romanov breeds; 136/Ala homozygotes appear to be resistant while heterozygotes can appear in both groups when sheep are experimentally infected (Maciulis *et al.*, 1992; Laplanche *et al.*, 1993). While these results are encouraging, not all naturally occurring scrapie isolates react in such a clear-cut way. The situation may be much more complex than it appears.

Experimental transmission of BSE to a variety of animals, including sheep, suggests that susceptibility is not entirely controlled by overall sequence similarity between donor and recipient PrP. Sheep, which have a PrP variant that differs from bovine PrP at codons 98, 100, 143, 146, 155, 158, 189 and 208, succumb to experimental BSE challenge in about 500 days (Goldman *et al.*, 1994) which is similar to the time for scrapie transmission in these sheep where no PrP sequence difference exists. However, sheep homozygous for a PrP gene encoding one additional substitution of Arg for Glu at codon 171 are entirely resistant to scrapie and BSE (Goldman *et al.*, 1994, 1996). Sheep heterozygous at codon 171 are resistant to both BSE and scrapie for at least 1800 days after challenge. According to Goldman *et al.* (1996) the combined effects of six amino acid differences between sheep and bovine PrP appear to be outweighed by a single additional change at codon 171 indicating that this may be a crucial region. These alterations and relationships are interesting, however correlation between any single feature of a PrP sequence and likelihood of transmissibility must be demonstrated experimentally.

There are at least 20 known mutations *within* the PrP gene sequence resulting in "spontaneous" PrP^{sc} formation. People who are homozygous at the polymorphic amino acid position 129 of PrP protein are predisposed to both acquired and sporadic CJD. The genotype conferring genetic predisposition to CJD upon exposure to environmental prions is Val homozygosity at 129 (Palmer *et al.*, 1991). Some GSS families have a mutation at 102 where Leu is substituted for Pro in the PrP (Prusiner, 1995). Two unrelated GSS families have the same double mutation: codon 178 (Asp/Asn) and codon 200 (Glu/Lys).

BSE has been experimentally transmitted (intracerebral inoculation) to macaques; these animals developed symptoms clinically similar to human vCJD (Lasmezas *et al.*, 1996). There are a number of differences between the PrP gene of humans and those of apes and monkeys which do affect their amino acid sequence, however the amino acids affected may lie outside the proposed helical domains (where they may not influence the stability of the helical domains) (Prusiner, 1995).

VI. PRION STRAINS

Two types of PrP^{sc} have been defined by size and glycosylation differences (Parchi *et al.*, 1996). Within these types, various "strains" may exist for a particular host species. There are at least 20 strains of scrapie which are differentiated by their incubation period, "lesion profile", and physical symptoms. A strain is determined, in part, by semi-quantitative evaluation of pathological damage: vacuolation is scored in nine regions of the brain. These scores, expressed graphically, are known as a lesion profile or "signature" (Bruce *et al.*, 1994). Strains can be transmitted in inbred mouse strains that are homozygous for a single PrP genotype; serial transmission results in no change in the "strain" characteristics (i.e. strain phenotype is unaltered). This implies that a specific PrP is converted to a specific PrP^{sc} strain which is distinct from other strains (Bessen *et al.*, 1995). The existence of scrapie strains has been difficult to explain based on the protein-only hypothesis. A number of investigators have suggested that prion protein is not the infective agent or that it does not act alone. A requirement of the protein-only model is that the "inheritance" of pathogen strain differences must be mediated by stable variations in PrP^{sc} structure rather than mutations in an agent-specific nucleic acid.

Prusiner (1995) proposed that prions can adopt multiple conformations: folded in one way, a prion might convert normal PrP to PrP^{sc} very efficiently giving rise to short incubation times; folded another way, it might be less efficient resulting in a longer incubation period. In addition, it is possible that one conformation might be attracted to neuronal populations in one part of the brain while another conformation might be attracted to neurons in another part, producing different symptoms.

In order to investigate the mechanism of scrapie strain propagation, Bessen *et al.* (1995) investigated whether the biochemical differences between PrP^{sc} from hyper (HY) and drowsy (DY) hamster prion disease could be transmitted. Both the HY and DY PrP^{sc} are post-translationally derived from the same PrP precursor; they are cleaved at different amino-terminal sites by proteinase K. Using a cell-free conversion system, addition of HY PrP^{sc} or DY PrP^{sc} produced "strain specific" PrP^{sc} from the same unglycosylated PrP precursor. The two distinct sets of protease-resistant PrP^{sc} products provide evidence that self-propagation of PrP^{sc} polymers having distinct three-dimensional structures could be the molecular basis of scrapie strains. The PrP^{sc} strains may be alternative conformations or packing arrangements of PrP^{sc} polymers (Bessen *et al.*, 1995).

Based on lesion profiles upon passage in mice, BSE in cattle seems to be caused by a single prion strain type with unusually persistent phenotypic

properties (following passage in other species) (Bruce *et al.*, 1991b, 1994). Based on the lesion profiles, many of the newly recognized animal prion diseases affecting cats (domestic and captive wild) and captive wild ungulates in the UK appear to have resulted from a direct transmission of BSE (through contaminated feed) rather than from transmission of scrapie (Bruce *et al.*, 1994). The BSE agent has been biologically "typed" using disease incubation times and neuropathological lesions in inoculated mice; BSE is a "scrapie-like" agent but is distinguishable from previously known strains of scrapie (Bostock, 1994). If BSE is associated with the new variant of CJD (vCJD) in the UK, the BSE "signature" should be detectable following passage in humans.

Recent evidence indicates that the TSE strain occurring in vCJD is not different from the strain responsible for BSE in cattle. Bruce *et al.* (1997) reviewed the data based on lesion profiles generated by injecting inbred mice with tissue containing BSE, CJD, vCJD and TSEs of other animals. They also report interim results of injection of vCJD and CJD into mice. Their data provide evidence that the same (TSE) strain is involved in both BSE and vCJD. Collinge *et al.* (1996) found a means of using the biochemical properties of the prion protein to trace the likely causes of vCJD. When the new prion protein chemical signature tests were applied to brain tissue samples from 26 human patients who had died from all four forms of CJD and compared with samples of tissue from laboratory animals who were experimentally infected with BSE, the patterns for familial, acquired and sporadic CJD were similar to each other, but they were different from the BSE pattern. However, the vCJD pattern was similar to the BSE pattern. Using a different approach, Hill *et al.* (1997a) came to the same conclusion by evaluating the glycoform profiles evident in different TSE strains. They focused on the fragment sizes and ratios of di-, mono- and nonglycosylated forms of PrPs known to be involved in different diseases. The pattern of glycosylation (glycoform profile) was maintained during transmission from species to species. The glycoform profile of vCJD is different from that of classical CJD but the same as that of BSE. Together, these studies provide powerful evidence that vCJD was contracted from BSE-infected materials; whether or not these infected materials were derived from the human food chain or from other sources is still not clear. On 2 October 1997, the editors of *Nature* (vol. 389) stated that the evidence "makes it highly likely that humans with vCJD contracted it by consuming meat from cattle infected with BSE." If that is the case, we can expect to see more cases of vCJD; however, because incubation time of vCJD is essentially unknown as are the doses and infectivity of materials to which people have been exposed, it is difficult, if not impossible, to predict when and how many. Using epidemiological data, known incubation times of CJD and Kuru, Cousens *et*

al. (1997) estimate somewhere from about 200 cases up to 13,000 cases depending on dose, infectivity, and time of exposure.

Based on advice from the Spongiform Encephalopathy Advisory Committee (SEAC), the UK government decided to point out the probable link between vCJD and BSE in March 1996, even though the evidence was scarce. A fair amount of scientific support materialized in October 1997, in the previously mentioned studies (Bruce *et al.*, 1997; Hill *et al.*, 1997a) to support the SEAC's original vCJD–BSE link.

VII. SPECIES BARRIERS TO PRION TRANSMISSION

The concept of a "species barrier" has long been thought to protect animals, including man, from diseases of other animals. Transmission of prion diseases from one mammalian species to another is often difficult, with only a small proportion of inoculated animals developing disease following an extremely long incubation period. Infection transmission from one species to another often requires large doses of infective tissue injected directly into the nervous tissues of the recipient species, a distinctly artificial system. Subsequent passage within the recipient species generally results in a reduction in incubation period (which eventually stabilizes) (Collinge *et al.*, 1996). The difference between host-to-recipient incubation time and recipient-to-recipient incubation time is often referred to as the "species barrier" (Pattison, 1965). Foreign host PrP^{sc} may be inefficient at inducing conformational change in primary recipient PrP resulting in a long induction period while sufficient titer builds up to result in disease. Upon passage from primary recipient to secondary recipient, most of the PrP^{sc} is the infectious isoform from the primary recipient PrP; it may be more effective at conformation change-induction resulting in build-up to high titer in a short period of time accompanied by short incubation period. This alteration in incubation period is a direct result of cycling within a species, particularly when the PrP amino acid sequences differ greatly between host and recipient (Howard, 1996; Collinge *et al.*, 1996).

The species barrier is not constant, but rather, varies from disease to disease and from animal to animal (Blanchfield, 1996). In general, the species barrier between animals and man are of quite a different order from that between different animals (Collinge *et al.*, 1996). There are some differences between species in the normal PrP sequence; however, there are also differences within a single species. These differences present some barrier to PrP^{sc} formation; the greater the difference, the greater the barrier. However, sufficient exposure can apparently overcome the barrier in many cases (Howard, 1996). The extent to which prion diseases can cross species

barriers depends on several factors including prion strain, prion dose, genetics of the recipient individual within a species, and method of introduction (oral vs. systemic vs. intracerebral vs. conjunctival) (Palmer and Collinge, 1993).

Whether or not a recipient is susceptible to prion infection is determined by both the prion inoculum (source, strain and dose) and the PrP gene of the recipient. For example, a single mouse prion isolate will produce disease after varying incubation times which depend on the mouse strain (*Sinc* genotype). The species barrier can be "overcome" if a transgene from the prion donor can be introduced into the recipient species (Collinge *et al.*, 1996).

While human and animal prion diseases have been experimentally transmitted to various monkey species, spontaneous occurrence of a spongiform encephalopathy in non-human primates has not been reported until recently. Bons *et al.* (1996) reported that in 1991 a previously healthy rhesus monkey became lethargic, developed mood changes (aggressiveness) and hid from its companions. The brain of this monkey showed spongiform changes and large vacuoles in the neurons. Immunohistochemistry showed distended nerve-cell processes surrounded with PrP-immunoreactive material similar to that seen in squirrel monkeys inoculated with brain material from patients with CJD. Immunological labeling of neurons in this monkey resembled those seen in the brains of patients with CJD. Similar results have been reported in macaques intracerebrally inoculated with brain extracts from cattle suffering from BSE, however histopathology revealed that the macaque disease resembled vCJD more closely than CJD (Lasmezas *et al.*, 1996).

A breeding colony of marmosets (n = 100) in the UK were fed pellets containing 20% ruminant-derived meat-meal protein from 1985 to 1996 (peak years of BSE in cattle). Adults weighing about 350 g ingested about 2 g meat-meal/day as a part of their normal diet. Over their 5–15 life spans, histopathological evaluation of brains revealed no evidence of neurodegenerative change (Ridley *et al.*, 1996). The level of BSE-infective materials contamination, if any, of the diet was unknown.

The species barrier resides, at least partially, in the amino acid sequences of host and recipient PrP: the more homology there is between the PrP^{sc} molecule and the PrP sequence of the recipient into which it is introduced, the more likely it is that the host will acquire prion disease. The mouse gene differs from the hamster gene at 16 codons out of 254; normal mice inoculated with hamster PrP^{sc} rarely acquire scrapie, however trangenic mice (those containing hamster genes) become ill within two months (Prusiner, 1995). The difference between sheep and bovine PrP is small (7 positions), while the difference between human and bovine PrP is large (30 positions).

However, certain regions of the PrP seem to be critical; homology in those regions may be more important in predicting transmission than overall homology (Prusiner, 1995). Krakauer *et al.* (1996) found two pairs of derived substitutions in genes coding for prions that were uniquely shared by cattle and hominoids. While drawing no conclusion from this, the authors note that based on the improbability of this occurring by chance, it likely has some biological significance (i.e. improving the efficiency of PrP function in these species). It is possible that these similarities may have predisposed humans to a strain of prion disease that occurred in cattle.

BSE titers as high as 10^7 have been reported in some mouse genetic lines may be only 10^4 in mice of different genetic lines. This would suggest an intra-species genetic barrier as high as 1:1000 (Collinge *et al.*, 1995, 1996). Based on this, the minimal barrier between species would be at least as high as the 1:1000 barrier within a species. A species barrier of 10^7 is considered large.

VIII. HUMAN RISKS AND CREUTZFELDT-JAKOB DISEASE

Four cases of CJD among dairy farmers in the UK occurred in 1994–95. However, high rates of CJD occur among cattle farmers in other European countries in which BSE is either rare or unreported (National CJD Surveillance Unit, 1995). These cases resembled typical sporadic CJD. This event was an alert to the UK to the possibility of BSE transmission to humans as vCJD. However, subsequent strain typing of the TSE occurring in the farmers revealed that it was not different from sporadic CJD but was quite different from vCJD (Hill *et al.*, 1997a).

The number of cases of CJD in the UK rose in the 1990s engendering concern because of the unusual cluster and characteristics of the CJD cases. The world-wide sporadic CJD incidence is roughly constant and is not different between meat-eating and vegetarian populations. CJD is rare and occurs with a wide variation in clinical presentation; sporadic cases with atypical presentation are very likely to escape clinical detection. Bruton *et al.* (1995) recently reported that 40% of prion disease cases identified neuropathologically (postmortem) had been "undetected" during life; most were found, retrospectively, to have had an atypical presentation including psychiatric symptoms.

As the number of cases of CJD increased and the distinction occurred between CJD and vCJD, vCJD was suggested to have originated from ingestion of high-titer BSE-containing materials in the mid-1980s before the UK SBO ban; this scenario accounts for an incubation period of

5–10 years. The shortest incubation period for a human prion disease (4 to 5 years) is for kuru (where no species barrier exists).

In 1996, the British Spongiform Encephalopathy Advisory Committee (SEAC) concluded that, "the most likely explanation at present is that these (vCJD) cases are linked to exposure to BSE before the introduction of the specified bovine offal (SBO) ban in 1989". At that point, the UK government stressed that there was still no proof of a definite link but accepted that the new evidence was cause for concern.

The risk of infection to humans is dependent on: (1) *both* between-species differences and within-species gene sequence variations; (2) dose of infective agent; and (3) route of introduction. A species barrier high enough to prevents cross-species transmission of BSE to man could exist. The dose required to transmit BSE from cattle to mice and a variety of other experimental animals are either known or being elucidated; the species barrier between cattle and man is essentially unknown.

In humans, the prion codon 129 polymorphism impacts susceptibility of individuals to CJD. Heterozygosity at codon 129 protects individuals from CJD. *vCJD patients have all been m/m genotype.* Transgenic mice with a human transgene (Val/Val at 129) developed prion disease when challenged with CJD. Mice expressing only human PrP (produced by breeding PrP null mice with human PrP mice) developed disease with a much shorter incubation time. Transgenic mice challenged with BSE appeared to produce mouse PrP^{sc}. Mice expressing only human PrP, then challenged with BSE, remained symptom-free at the conclusion of the study, 60 days longer than the time normally required for the appearance of CJD in mice of this genotype (Collinge *et al.*, 1995). The question now is what would be the outcome of BSE challenge to transgenic mice containing human transgenes with Met/Met at codon 129?

Administration route is critical to infective dose. In the mouse, administration of scrapie via the oral route is very inefficient: Scrapie infectivity is about 10^7 LD_{50}/g wet tissue when administered by the intracerebral route (infectivity seems about the same for BSE when administered to mice) (Kimberlin, 1991). The murine oral or gastric LD_{50} = 100,000 intracerebral LD_{50} dose (Kimberlin and Walker, 1978). Introduction via the subcutaneous route seems to be intermediately efficient.

Because prion diseases lack immunological involvement in host animals, those at immunological risk (infants, children, hospital patients, pregnant women and people taking immunosuppressive drugs) are likely to be *no more* susceptible than adults.

Exposure to BSE-infective material is a critical question if it can breach the cattle-to-man species barrier. Prior to the 1989 SBO ban in Great

Britain, an estimated 446,000 BSE-infected animals likely entered the food chain and were consumed (either directly or as processed products). Between 1989 and 1995, when there was an estimated 50% compliance with the SBO ban, an estimated 3–5% of the animals (283,000) that entered the food chain were infected (Andersen *et al.*, 1996a). With the exception of brain, spinal cord and related tissues, retina, distal ileum of calves, and the buffy coat of lymphocytes in a few studies, all other tissues and secretions from confirmed BSE cattle have failed to transmit the disease when injected into or fed to mice. In particular, milk and meat have not transmitted the disease in experimentally infected animals, and the World Health Organization (WHO) considers that milk and milk products, gelatin and properly rendered (protein-free) tallow (even from the UK) were safe for human consumption.

However, scientific opinion is not unanimous about the current safety of beef particularly in light of findings published by Bruce *et al.* (1997) and Hill *et al.* (1997a) showing that the BSE strain type is not different from that identified in vCJD. Whatever the risk, it should have been greater for UK consumers before the 1989 SBO controls were introduced. The risk to the US population is essentially zero since there have been neither confirmed BSE cases nor importation of beef or beef products from BSE-positive countries since 1985. Because of the lengthy incubation period (in humans) for CJD, it will be some time before even tentative estimates can be made of the number of people that are likely to have contracted vCJD from beef before 1989, assuming that transmission could and did occur. Actual exposure remains an open, and perhaps unanswerable, question.

IX. INFECTIVITY

The degree to which a tissue can transmit disease is expressed in infective units (IU); one IU is the minimum amount (of that tissue) required to infect another animal *of the same species* by intracerebral inoculation. TSE infectivity has been measured by mouse bioassay. Infectivity is calculated in $\log_{10}$ of infective units/g (Dealer, 1993). Using sheep as an example, the infectivity of muscle, kidney (about 2.5) < adrenal < pituitary < nerve, lymphoid < spinal cord, gut < brain (about 7) (Dealer, 1993). Infectivities of TSEs for humans appear to be similar (Brown *et al.*, 1994b). It is relatively easy to transmit BSE to mice, but even easier to transmit BSE to calves because of the lack of a species barrier. Wilesmith (1996) reported that experimental intracerebral inoculation of BSE-infected brain tissue from cows into calves has resulted in a detection level 1000 times more

sensitive than the mouse bioassay. Tissues not found to be infective by mouse bioassay may prove to be infective via intracerebral inoculation into calves. Neither lymph nor spleen (both negative by mouse bioassay) have proven to be infective by calf assay; however meat and milk have not been evaluated.

Wuthrich (1996) stated that most TSEs have about 10^7 to 10^8 IU/mg tissue (which he translates to 1 IU per 10^5 PrP^{sc} molecules). Because some tissues are much more infective than others, they transmit disease at lower doses, and they will require more reduction in infectivity (stronger treatments) to inactivate infectivity.

Infectious dose is usually expressed in terms of LD_{50} – the amount of agent required to kill 50% of the population exposed to it. Scrapie infectivity is about 10^7 LD_{50} /g wet tissue administered intracerebrally which is similar for BSE (Collinge *et al.*, 1995). The infectious dose varies depending on the route of exposure. Heaphy (1996) states that 1 gastric $LD_{50} = 10^5$ i.c. LD_{50} (measured by comparing BSE titers in mice) (Kimberlin and Walker, 1978). BSE appears to have between 10^7 and 10^{10} LD_{50}/g wet tissue by intracerebral injection (mice).

X. INACTIVATING THE INFECTIOUS AGENT

Prion infectivity is extremely difficult to destroy. Prions remain infective after: sterilizing levels of radiation, exposure to formalin, dry ashing at 360°C (Marsh and Bessen, 1993; Brown *et al.*, 1990b), UV light (2500 nm) (Haig *et al.*, 1969), extremes of pH, non-polar organic solvents (methanol, hexane, petroleum ether), burying in the soil for years, passing through 0.1 μm filters (2.2 μm filters remove bacteria), and porous load autoclaving at 134°C for 60 min (Taylor *et al.*, 1994). Purified scrapie preparations maintain their infectivity titer after treatment with proteases, amylases and nucleases (Brown *et al.*, 1990b).

Taylor *et al.* (1994) and Taylor and Fernie (1996) reported that infectivity in homogenates of BSE-infected bovine brain was no longer detectable after exposure to sodium hypochlorite containing 16,500 ppm available chlorine for 120 minutes; neither sodium dichloroisocyanurate nor 2 *M* NaOH provided complete inactivation. However, infectivity was destroyed by 1 *M* NaOH at 55°C. The scrapie prion is inactivated by treatment with saturated phenol, 10% SDS (sodium dodecyl sulfate) with a minimum of 30 minutes of exposure. Iodine, hydrogen peroxide, and formaldehyde have little or no effect with up to 24 hours of exposure (Brown *et al.*, 1982, 1990a). Urea (8 *M*), 1 *M* KSCN, psoralen, and NH_2 have no effect (Heaphy, 1996). One hour in an autoclave at 121°C reduced high titer (>10^{10} LD_{50})

preparations of scrapie-infected hamster brain by about 7.5 LD_{50}/g, leaving about 2.5 log LD_{50}/g of residual infectivity (Brown *et al.*, 1982). While some loss of infectivity occurs at temperatures above 100°C, even 30 to 60 minutes at more than 140°C does not always result in total inactivation meaning that traditional autoclaving may be insufficient with high titer materials (Brown *et al.*, 1990b; Marsh, 1991). Brown *et al.* (1990b) suggest that treatment with formaldehyde before autoclaving (commonly used with laboratory tissue samples) actually preserves infectivity, possibly by intramolecular formaldehyde cross-stabilization; treatment after autoclaving reduces or inactivates scrapie infectivity completely. Recommended treatment of contaminated equipment, tissues, etc. includes treating materials with 1 *N* NaOH (prior to sterilization) and flushing contaminated surfaces with 1 *N* NaOH or 5% hypochlorite (Brown *et al.*, 1990b).

Taylor *et al.* (1995b) rendered BSE-infected brain tissue, bovine or porcine intestine and bovine bone; meat and bone meal (MBM) were produced from the rendered materials then infectivity was evaluated. Continuous atmospheric processing of materials with natural fat content or with high fat content to temperatures of 112–122°C for times ranging from 20 to 57 min did not completely inactivate BSE infectivity. The implication is that some rendering processes may be inadequate to rid materials of all infectivity.

Scrapie has remained endemic in Iceland even though all sheep in the affected area were removed, farms were cleaned and disinfected and no sheep were reintroduced for three years; reintroduced sheep developed scrapie (Georgsson and Sigurdarson, 1995). A similar situation for endemic CWD has occurred in Colorado (Williams and Young, 1992). Survival on the premises, in soil or in other indigenous species may not bode well for total eradication of TSEs anywhere.

XI. BY-PRODUCTS

A. TALLOW

Tallow is the hard fat rendered from the fatty tissues of cattle that is removed during processing of beef. Inedible tallows and greases, derived from animals or animal parts deemed unfit for human consumption, are major by-products of the meat packing industry. Edible tallows come only from specified organs of healthy bovine animals (Codex Alimentarius, 1969). In the UK, the majority of inedible tallow and grease are removed in an initial low temperature "melting" step then the greaves are further processed. Tallows are produced by fat release from fatty tissue using both

temperature and cell rupture. Rendering may be "wet" or "dry". During wet rendering, fatty tissues are heated in water usually at temperatures lower (82–88°C) than those used in dry rendering. Most of the inedible tallows and greases are produced by dry rendering; however, lower temperatures can be used for dry rendering if it is accomplished at elevated pressures. Fatty tissues are heated in steam-jacketed cylinders containing internal rotating blades. Moisture is cooked out, fat is released and the remaining mixture is strained to remove remaining solids. The solid material is dried in continuous cookers to produce meat and bone meal (Dugan, 1987). The US rendering industry voluntarily excluded sheep from entering the rendering stream in 1989 (Franco *et al.*, 1996); an estimated 85% of renderers complied with the voluntary ban.

While the UK substantially modified its rendering practices during the 1970s and 1980s, eliminating the solvent extraction step, the US did not, primarily because solvent extraction was not being used in the US. "Fat melters" (knackers) do not exist in the US and the USDA does not allow that type of resultant material (essentially raw) to be used in animal feed (Anderson *et al.*, 1996b). US rendering depends on high temperature treatment of fatty tissues. In addition, subsequent to the APHIS Surveillance program, if an animal has the head removed for histopathological evaluation (due to apparent neurological symptoms), the remainder of the animal may not be rendered (Franco *et al.*, 1996).

The EC originally banned importation of almost all beef products into member states, but partially lifted the ban (96/362/EC) in June 1996, permitting export of UK bovine semen, and setting forth conditions under which gelatin, tallow, amino acids and peptides could be exported. Tallow and tallow products had to be produced from material from animals fit for human consumption (Art. 2, Decision 94/382/EC). Products derived from tallow via hydrolysis at 250°C or higher were also permitted. This Decision came into force on 15 March 1997 but was replaced by the Bovines and Bovine Products (Despatch Prohibition and Production Restriction) Regulations 1997 requiring registration of plants producing these products for export. Three UK plants are registered under the new regulations; they are subject to weekly inspections. Their tallow is currently the only UK-produced tallow which can be used in food, feed, pharmaceutical, medical and cosmetic products.

The EC Scientific Veterinary Committee concluded that a rendering procedure using 133°C for $\geq$ 20 min is the minimum time-temperature required to produce safe meat and bone meal, but that even this system cannot guarantee complete removal of TSE agents (Commission Decision, 1997).

B. GELATIN

About 65% of the gelatin produced world-wide comes from hidesplits, connective tissue and the bones of cattle; the remainder comes primarily from swine (Schrieber and Seybold, 1993). During gelatin production, bones are splintered into very small pieces (<12 mm), fat content is reduced (< 2%) with hot water (degreasing), then bone is dried for a minimum of 30 min at ≥ 100°C. Bone is demineralized (of phosphate) starting with dilute HCl, increasing to 4% HCl over 5 days, leaving only the collagen structure. The final pH (1.5) corresponds to about 0.1 *M* HCl (Heynkes, 1996). Brown *et al.* (1986a) demonstrated that 1 *M* HCl was insufficient to completely inactivate the scrapie agent but did reduce the concentration. More than 90% of bovine gelatin is alkalyzed at pH 12.5 (<0.1 *M* NaOH) for 50 days with NaOH; alternatively 0.3 *M* NaOH for 14 days can be used to open cross linkages between collagen molecules (Heynkes, 1996). The resulting gelatin is sterilized at 140°C for 4 sec; higher temperatures (or longer exposure time) irreversibly damage the functionality of the gelatin (Heynkes, 1996).

Manzke *et al.* (1996) reported that the standard hot water degreasing step resulted in a 100-fold reduction in nerve-specific proteins from bovine brain tissue. Pupkis (1996) reported that infectivity of scrapie tissue could be reduced from $10^{8.2}$ LD_{50}/ml to $<10^{2.4}$ by the acid treatment and $<10^{3.4}$ by the alkaline treatment used to manufacture gelatin; length of exposure to acid or alkali had little or no effect. Brown *et al.* (1990a) demonstrated that autoclaving for 18 min at 134–138°C was insufficient to inactivate BSE agents completely. Scrapie-infected hamster brains remained infectious after 1 hr at 360°C. In short, high titer TSE-infected materials remain infective when exposed to 10 times the acidity, 20 times the alkalinity and 220°C over the processing temperature used for gelatin; however, all of these treatments do reduce infectivity (some by many orders of magnitude) (Brown *et al.*, 1990a; Pupkis, 1996). Taylor *et al.* (1994) reported that UHT sterilization used in gelatin manufacture would reduce any residual infectivity at least 10-fold and more likely 1000-fold. The combined infectivity reduction resulting from degreasing, acid demineralization, alkaline treatment, and sterilization has been estimated to be on the order of 10^9 (Blanchfield, 1996). Hidesplits, connective tissue and bones of BSE-infected animals are far less infectious than central nervous system tissues, and the presence of 15 kg of raw material from one (infected) cow is diluted up to 100 times in large production batches. While infectivity may be substantially diluted, it is still a crucial consideration.

The EC has alternately banned and permitted the export of gelatin from the UK. In May 1996, the European Commission required changes in the

manufacturing process such that gelatin would be "safe"; when it became clear that the process could not be so modified and still produce a functional product, the EC withdrew permission for the export of UK gelatin (May, 1997). The three companies (German, Belgian and British) that produce gelatin in the UK have not used British raw materials since Spring 1996; their manufacturing processes are similar to those used elsewhere. The EC originally banned beef and most bovine products from UK export into EU member states. The Standing Veterinary Committee determined that infectivity was substantially reduced by the gelatin manufacturing process. Commission Decision 96/362 (June 1996) permitted the resumption of (UK) exports of gelatin provided it is produced in a process which ensures that all bone material is degreased, acid treated at a pH <1.5 for at least 4 days, followed by an alkaline treatment that uses either lime at pH >12.5 for at least 45 days or 0.3 *M* NaOH for 10–14 days followed by heating to between 138 and 140°C. Source materials must be fit for human consumption. In April 1997, the EU's scientific committee recommended that the ban (on gelatin) be reimposed, which it was in May 1997 (MAFF, 1997a).

Because some doubt remains as to the BSE-neutralizing capabilities of the gelatin manufacturing process, the FDA issued a "Guidance for Industry. The Sourcing and Processing of Gelatin to Reduce the Potential Risk Posed by Bovine Spongiform Encephalopathy (BSE) in FDA-Regulated Products for Human Use" to ensure safety (Friedman, 1997). It is based on a three-tiered risk management scenario; the tiers consist of sourcing, processing and use (1994). In April 1997, the FDA's Transmissible Spongiform Encephalopathy Advisory Committee recommended that gelatin from BSE-positive countries not be imported into the US (FR, Vol. 62, No. 74, pp. 18728–18730). The committee said that while there is no evidence that BSE can be transmitted to humans from gelatin, the FDA should be allowed to regulate it, if necessary. However, on 2 October 1997, the FDA said that there is no scientific basis for banning the use of gelatin in consumer products intended for oral consumption or cosmetic use if the gelatin is derived from countries reporting cases of BSE. FDA-regulated products that are injectable, for use in or on the eye, or implanted should not be manufactured from gelatin produced from cattle from BSE-positive countries.

XII. DIAGNOSTIC TESTS AND SURVEILLANCE

There is no widely available laboratory test able to reliably detect the TSEs in live animals for routine diagnosis. Veterinary pathologists confirm BSE

by postmortem microscopic examination of brain tissue (Groschup *et al.*, 1994). The search for practical preclinical diagnostic tests for the SE diseases has been a primary research goal. Most of the work has focused on detection of infectivity (mouse bioassay) or molecular changes associated with the infection (detection of SAF or PrP^{sc}). Scrapie associated fibril proteins can be detected using anti-SAF antibody even in nonpurified tissue preparations (Rubenstein *et al.*, 1986). However, these tests are both postmortem detection methods.

Infectivity of TSE-suspect materials is accomplished primarily by mouse bioassay (Schreuder, 1994). Genetically susceptible (inbred) mice have been developed to assess scrapie infectivity titers by injecting limiting serial dilutions; however, not all scrapie isolates produce the same results. Different isolates can show different incubation periods upon sub-passage in the same strain of mice indicating that the isolate has crossed the species barrier (Schreuder, 1993). These isolates also show different histopathological lesions in mice which forms the basis of strain typing.

Extensive testing of bovine materials by mouse bioassay has failed to detect BSE in muscle tissue or milk. Intracerebral inoculation with tissues from BSE-infected animals has resulted in no detectable infectivity in lymph tissue or spleen. Lymph and spleen are suspect tissues because they are infective in sheep. Results using more sensitive calf tests on meat, milk, tallow and gelatin have not yet been reported.

Tests to detect SAF or PrP^{sc} outside of the central nervous system would be very useful in live animal pre-clinical diagnosis. Both SAF and PrP^{sc} have been detected by Western blot in the spleens and pooled lymph nodes of scrapie-infected mice, but neither have been detected in white blood cells (Doi *et al.*, 1988). Positive results using Western blot on spleen and lymph nodes as well as CNS tissues have been reported for clinically ill, naturally infected sheep, but less dependably with experimentally infected, asymptomatic sheep (Ikegami *et al.*, 1991). Grathwohl *et al.* (1996) reported a detection technique where PrP^{sc} from spleen and lymph nodes of recently infected (scrapie) mice in which tissues were homogenized, digested with collagenase, and PrP^{sc} was salted out prior to detection by Western blot.

TSE detection in peripheral tissues in human CJD has been somewhat successful. Meiner *et al.* (1992) detected PrP^{sc} in cultured fibroblasts and in monocytes in CJD patients; the PrP^{sc} in monocytes was more proteinase-resistant than that in fibroblasts. Buffy coat from blood cells of patients with CJD is known to transmit TSE to rodents (Tateishi, 1985).

Despite publicity about a possible spinal-fluid based detection method

(Bruce *et al.*, 1997) the test is not specific for BSE. The presence of the 14-3-3 brain protein in cerebrospinal fluid indicates nerve cell death. TSE infected animals test positive for 14-3-3 protein, however positive tests may also result from inflammatory brain disease (BSE is not inflammatory), herpes, encephalitis or coronary infarction.

Van Keulen *et al.* (1995) developed antibodies to selected synthetic PrP-based peptides. Schreuder *et al.* (1996) used these antibodies to detect PrP^{sc} in the tonsils of a group (n = 55) of sheep naturally infected with scrapie (clinically positive for scrapie based on immunohistochemistry). This antibody was then used to detect scrapie in sheep during the preclinical phase of infection in susceptible sheep. Schreuder *et al.* (1996) were able to detect infection in less than half (10 months) the expected incubation period (25 months) using tonsil biopsy. The results of the biopsies were subsequently confirmed with immunostaining.

A monoclonal antibody specific for the abnormal version of the prion protein has been reported in *Nature* (Korth *et al.*, 1997). Conformation differences between PrP and PrP^{sc} are evident from increased beta-sheet content and protease resistance in PrP^{sc}. A monoclonal antibody (15B3) specifically precipitates bovine, murine and human PrP^{sc} but not PrP suggesting that it recognizes an epitope common to prions from different species. Three polypeptide segments in PrP have been mapped: segments 2 and 3 of the epitope are near neighbors and segment 1 is located in a different part of the molecule in recombinant mouse PrP. The PrP^{sc}-specific 15B3 epitope may recognize PrP^{sc} as distinct from PrP by spatial proximity of all three 15B3 segments.

XIII. CONTROL MEASURES IN THE UNITED KINGDOM

Agricultural officials in the UK have taken a number of actions to eradicate BSE and to safeguard the public health, including (1) making BSE a notifiable disease, (2) prohibiting the inclusion of ruminant-derived proteins in farm animal feed, (3) destroying all animals showing signs of BSE, (4) prohibiting the consumption of milk from affected or suspect cows by either animals or humans (except dam to calf), (5) stopping human and animal consumption of certain bovine tissues, and (6) selectively culling those animals most likely (>30 months of age) to be incubating the disease. These actions have been taken over the time period from 1988 to 1997 as scientific information emerged.

In July 1988, the United Kingdom Minister of Agriculture, Fisheries and Food (MAFF) banned the use of cattle with clinical BSE signs from entering the food supply (human or animal). In 1989, the Specified Bovine

Offal (SBO) ban eliminated a variety of potentially infective tissues from movement into the food chain. These included brain, spinal cord, tonsil, thymus, spleen and intestine from animals over 6 months of age. The SBO ban amounted to a statutory ban on ruminant-to-ruminant feed supplementation (Anderson *et al.*, 1996b); these materials were actually banned from ruminant feed in 1990 (MAFF, 1998). The list of specific bovine offals (SBOs) banned for use in the food chain was later extended to cover a wider range of potentially infective materials including the whole head and vertebral columns. All are required to be stained with a bright colored, indelible dye (brilliant blue V (E131)). Slaughterhouse requirements were tightened to be sure SBO's were eliminated. Milk from cattle suspected of having BSE was destroyed.

In 1989, to prevent BSE spread to EU Member States, the EC banned export of British cattle over 6 months of age (Decision 94/474/EC (OJEC L 194 p. 96)). Calves of BSE-free parents could be exported only if marked then subsequently slaughtered before 6 months of age. The EC prohibited all Member States from feeding meat and bone meal to ruminants (Decision 94/381/ED (OJEC L 172 p. 25)).

The SBO in Britain led to a dramatic decline in new BSE cases in cattle. Cases peaked in 1992 and 1993 at over 36,000 and 34,000 cases in Great Britain. Confirmed case numbers had declined to 24,000 by 1994, to 14,000 in 1995, to 8,000 in 1996, to < 3,000 in 1997 (Andersen *et al.*, 1996a; MAFF, 1998). BSE peaked in 1991 for 3-year-old cattle, in 1992 for 4-year-old cattle, in 1993 for 5-year-old cattle, in 1994 for 6-year-old cattle and in 1997 for 7-year-old cattle (Anderson *et al.*, 1996a). The time lag between control measures and improved health status was due, in part, to farmers using up their stocks of cattle feed but mainly to the incubation period of the disease.

In November of 1995, the UK MAFF stated that the measures taken were recognized as being sufficient (to reduce BSE and protect the human population) by WHO, the International Veterinary Organization (the Office International des Epizooties) and the EC Scientific Veterinary Committee. The independent Spongiform Encephalopathy Advisory Committee (SEAC) which advises the UK Government on all aspects of BSE and CJD endorsed the measures and stated that in its view there was no evidence of a link between BSE and vCJD.

In January 1996, MAFF announced the following measures aimed at eradicating BSE via the animal feed transmission route: (1) carcasses of animals more than 30 months of age must be deboned in licensed plants under the supervision of the Meat Hygiene Service, and the trimmings classified as SBOs; (2) the use of ruminant meat and bonemeal in *all farm animal feed* (including that for horses and farm-raised fish) was prohibited

in April 1996. This resulted in the disposal of 11,000 metric tons of MBM and feed. By February 1997, <2% of feed in feedmills tested positive for mammalian protein (MAFF, 1998). No changes in current food safety advice were recommended at that time. Gelatin and tallow produced from non-SBO bovine material (under the direction of the Medicines Control Agency) were considered safe for pharmaceutical and food use; however MAFF made known its intention to review both in May 1996.

In May 1996, the Specified Bovine Offal (SBO) ban was changed to the Specified Bovine Material (SBM) removal requirement which specifies the whole head of bovines as SBM. In addition, meat from animals over 30 months of age could not be sold for human consumption. These animals (+30 months) were to be slaughtered, rendered and incinerated; this scheme is the "Over Thirty Month Slaughter Scheme" (MAFF, 1998). Between July 1996 and March 1997, 1,300,000 cattle were slaughtered in this program. This represents the cattle at highest risk so these actions were expected to reduce BSE cases by 10–15% in addition to the 40% yearly reduction reported following the SBO.

Therfore two cattle culls were running in the UK. One for BSE-infected or related animals; these animals were incinerated at MAFF plants. The second cull for those animals over 30 months which (if still alive and asymptomatic) should not have BSE; these animals were also being incinerated. Incineration occurs at 850°C for 2 seconds.

Many countries suspended imports of British beef following the announcement about the possibility of a link between BSE and vCJD. Most have not resumed importation.

The EC rendered a decision (Commission Decision, 1997) stating that in order to be imported into the Community, products of animal origin intended for food or feed must be accompanied by the required certificate supplemented by a declaration signed by competent authority of the country of production saying that the product does not contain, and is not derived from specified risk material (defined in Commission Decision 97/534/EC) or mechanically obtained material from the vertebral column of bovine, ovine or caprine animals. The decision, originally to go into effect 1 January 1998, was extended to March. This decision blocked import even from countries that have never had a case of BSE.

Based on longterm "pathogenesis experiments" conducted by MAFF (1997b) laboratories suggesting that dorsal root ganglia and bone marrow could transmit BSE, the UK Beef Bones Regulations of 1997 were enacted on 16 December 1997. These regulations required the deboning of all beef, regardless of country of origin, coming from cattle over 6 months, before it is sold to consumers. It prohibited the sale, to the ultimate consumer, of any bone-in beef or product containing an ingredient which was derived

from these bones; bones were to be rendered (in an approved plant), then destroyed by incineration or, under certain circumstances, buried or burned. These bones could still be used to prepare technical and pharmaceutical products.

XIV. PREVENTION OF BSE IN THE US

Meat imported into the US must be produced under standards *equivalent to those for meat produced inside the US* for safety, wholesomeness and labeling accuracy. The USDA Food Safety and Inspection Service (FSIS) has authority over all products from cattle (and other meat species) including processed products containing more than 3% raw meat. Imported meat must originate in countries and plants approved to export to the US. Approval requires an evaluation of the country's inspection system which includes a document review and an on-site review that focuses on five risk areas, facilities, laboratories, training programs, and in-plant inspection systems. Some 40 countries are approved to export to the US; the largest exporting countries are Australia, Canada and New Zealand. The US imports less than 10% of the domestic meat supply. To protect US livestock, APHIS restricts some products from entering the US because of animal disease conditions in the countries of origin; however, the meat from those countries could be shipped to the US if it were commercially heat-processed according to APHIS requirements.

Animal products (meat, hides, dairy products, etc.) are restricted if they originate in a country that has a disease status different from that of the United States. Animals and animal products must be accompanied by a health certificate issued by an official of the exporting country which is acceptable in the US. APHIS has failed to allow importation of live ruminants or their products (semen, embryos), by denying health certificates, since BSE was first recognized (1985) (USDA, 1996). In July 1989, USDA APHIS *banned the importation* of live ruminants and restricted some ruminant products from countries where BSE is known to exist (9 CFR 94.18). Products derived from ruminants, such as fetal bovine serum, bonemeal, meat and bonemeal, bloodmeal, offal, fats, and glands, cannot be imported into the US from these countries, except under a special permit for scientific research purposes (9 CFR 95.4). FSIS reports that there have been no beef imports from the UK since 1985 (USDA APHIS, 1998). In December 1991, APHIS restricted importation of ruminant meat and edible products and banned most by-products of ruminant origin from countries known to have BSE (56FR 63868, 56 FR 63869). Those countries include Belgium, France, Republic of Ireland, the Netherlands, Oman, Portugal, Switzerland

and the United Kingdom (Great Britain, Northern Ireland, and Falkland Islands). Prior to 1989, these products were restricted by not issuing permits. The regulation requires that *all* imported meat and edible products from ruminants in the bovidae family which are to be used for human or animal consumption must be deboned, must have visible lymphatic and nerve tissue removed, must be obtained from animals which have undergone a veterinary examination prior to slaughter, and must *be obtained from ruminants which have not been in any country in which BSE has been reported during a time period when that country permitted the use of ruminant protein in ruminant feed.*

The FDA has jurisdiction over cosmetic products, bovine-derived materials intended for human consumption as dietary supplements, and animal-derived human food (except meat). In August 1994, the FDA recommended that firms that manufacture or import dietary supplements and cosmetics containing specific bovine tissues (primarily nerve, glandular and intestinal) ensure that such tissues do not come from cattle born, raised or slaughtered in countries where BSE exists. This recommendation was not extended to dairy products or gelatin (9 CFR 95.4).

In April 1996, the World Health Organization made several recommendations (WHO, 1996):

1. No part of any animal with signs of TSE should enter any food chain, human or animal.
2. All countries should establish continuous surveillance and compulsory notification for BSE based on recommendation of the Office International des Epizooties (OIE).
3. All countries should ban the use of ruminant tissues in ruminant feed.
4. There is no evidence that milk or milk products transmit BSE; these products are considered safe, even in countries with high BSE incidence.
5. Because of the processing practices used to produce them, gelatin and tallow are considered safe for human consumption.
6. Medicinal products, because they may be injected, must be handled according to specific, previously set forth (1991) guidelines. Materials destined for the pharmaceutical industry must come from countries with a surveillance system in place and which report either no or sporadic cases of BSE.

Although no BSE infectivity has been detected in embryos, semen, or reproductive tissues of BSE-affected cows and bulls, a series of protocols based on the recommendation of the Office of International Epizooties for these materials are in effect in the US (USDA APHIS, 1998).

In the US, all cattle presented for slaughter undergo antemortem

inspection by FSIS for signs of central nervous system impairment. Any animal exhibiting neurological signs during this inspection is condemned; the meat is not permitted for use as human food, no part of the animal may be rendered, and brains are submitted to the USDA National Veterinary Service Laboratory (NVSL) for evaluation.

In 1986, APHIS established a BSE-surveillance program in the US and provided specialized training for APHIS veterinarians. Veterinary pathologists and field investigators also received training from UK veterinarians in BSE diagnosis. These pathologists examine brain tissue from cattle over two years of age that show signs of neurological disease; these animals would be the most likely group to be positive for BSE. In 1990, more than 60 veterinary diagnostic laboratories throughout the US began participating in the BSE Surveillance Program. USDA FSIS officials notify APHIS of cattle having neurological signs at slaughter. State diagnostic laboratories and public health officials also submit the brains of rabies-negative cattle to NVSL for testing. BSE is a notifiable disease in the US (Title 9, CFR, Parts 71 and 161). In addition to international importation restrictions, APHIS has increased surveillance efforts to detect BSE if it is accidentally introduced into the United States. More than 250 APHIS and State veterinarians specially trained to diagnose foreign animal diseases regularly conduct field investigations of suspicious disease conditions.

As of January, 1999, more than 7000 brains from throughout the US had been received at NVSL, and no evidence of BSE has been detected. Nearly 1000 of these brains have also been tested by immunohistochemistry for prion protein; it has not been detected in any of these specimens. Based on concerns related to nonambulatory "downer-cows" which are not presented for slaughter and therefore, not available for USDA inspection, these animals were included in TSE-BSE evaluations also. Based on a quantitative assessment performed by APHIS in 1993, if a TSE exists in nonambulatory cattle, it must be rare. USDA's Agricultural Research Service concluded that unidentified TSE could not exist in cattle at a prevalence greater than 1 infection per 975,000 cattle (statistical lower limit of detection) if it exists at all in the US (USDA APHIS, 1998). As part of increased surveillance for BSE, APHIS veterinarians traced 499 head of cattle imported from Great Britain between 1981 and 1989 (before the ban on imports went into effect) to check their health status. No signs of BSE have been found (AABP, 1996).

If the hypothesis that the origin of BSE is related to feeding rendered scrapie-infected sheep materials is true, the US should be at low risk of a "spontaneous" BSE epidemic. UK sheep outnumber cattle 3 to 1, while US cattle outnumber sheep 10 to 1.

In October 1996, the federal Centers for Disease Control and Prevention (CDC) released a report on the incidence of CJD in the US from 1979 to 1994. The CDC found no cases of vCJD in the US and the incidence of CJD overall was slightly less than 1/1,000,000 population annually (Anon, 1996).

Proposed rules to limit the use of ruminant-derived meat and bone meal in ruminant feeds (similar to the British SBO) were published in the Federal Register (3 January, Vol. 62, No. 2, 551–583) in 1997. A voluntary ban on the use of rendered products from adult sheep in animal feeds had been in effect since 1991 (AABP, 1996). The proposed ban was designed to limit potential spread of prion disease in US cattle from any spontaneous cases or from species in the US which are known to have prion diseases (deer, elk, mink). The final rule banning the use of mammalian protein (excluding blood, blood products, gelatin, processed meat products, milk products and porcine protein) from use in ruminant feeds was published 5 June 1997 (Vol. 62, 108).

XV. IMPLICATIONS

The safety of the US food supply has become a national priority: politically, economically and scientifically. Policy decisions are made based on the best data available at the time. The specter of a disease like BSE, its human health, animal health, and economic impact, looms large in the minds of many in agriculture, the food industry, the human and animal health industries, and government. The US has taken a very active approach to avoid bringing BSE into this country, to avoid developing a similar problem in our own animal agriculture industry and to protect consumers from any possible human spongiform encephalopathy risks that might be related to BSE. Because of the limited understanding of the infectious agents, routes of transmission and mechanisms of inactivation at the onset of the UK BSE epidemic, policies have been changing on a monthly basis as more information becomes available. Public trust (in the European Union member states and in the UK) was eroded by the apparent shifting sands of health vs. economic policies, when in fact, risk analyses often became outdated by the time the risk factors were written into policy. No cases of BSE have been identified in the US and, so far as we know, any material or product shown to transmit the disease has been banned for 10 years. However, as with any aspect of food safety, vigilance is imperative.

From the scientific perspective, the prion concept of disease transmission has infiltrated many areas rapidly and intensively, bringing together a

wide array of disciplines from human medicine to wildlife biology, to bring their expertise to bear on a devastating problem. In addition, the prion concept is a fascinating one, apparently defying many of the scientific principles we learned in school from "one genotype/one phenotype" to "heat denatures proteins". We can assume similar information will continue to be forthcoming. For the time being, we base our decisions on the best science has to offer *today*.

Currently, no cases of BSE have been identified in the US and no products have been imported that appear to pose potential risk to either human or animal health. We can assume that US beef and beef products are safe.

DEFINITIONS AND ACRONYMS

APHIS:	Animal and Plant Health Inspection Service
ApoE:	apolipoprotein E
BSE:	bovine spongiform encephalopathy
CJD:	Creutzfeld-Jakob disease
CNS:	central nervous system
CWD:	chronic wasting disease
DRG:	dorsal root ganglia
EC:	European Commission
EU:	European Union
FFI:	fatal familial insomnia
FSE:	feline spongiform encephalopathy
GSS:	Gerstmann-Straussler-Scheinker syndrome
ic:	intracerebral
IU:	infectivity units
LDL:	low density lipoprotein
LD_{50}:	lethal dose to 50% of animals tested
MBM:	meat and bone meal
MAFF:	Minister of Agriculture, Fisheries and Food
NVSL:	National Veterinary Service Laboratory
OIE:	Office International des Eqizooties
po:	per os (by mouth)
PrP:	normal prion protein
PrP^{sc}:	scrapie isoform of prion protein
SAF:	scrapie-associated fibrils
SE:	spongiform encephalopathy
SBO:	specified bovine offal
SBM:	specified bovine materials
SEAC:	Spongiform Encephalopathy Advisory Committee
TME:	transmissible mink encephalopathy

TSE:	transmissible spongiform encephalopathy
UK:	United Kingdom
USDA:	United States Department of Agriculture
vCJD:	new variant CJD
WHO:	World Health Organization

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