Evaluation of the Potential for Bovine Spongiform Encephalopathy in the United States

Joshua T. Cohen
Keith Duggar
George M. Gray
Silvia Kreindel

Harvard Center for Risk Analysis
Harvard School of Public Health

Hatim Abdelrahman
Tsegaye HabteMariam
David Oryang
Berhanu Tameru

Center for Computational Epidemiology
College of Veterinary Medicine
Tuskegee University

November 26, 2001
Revised October, 2003

Acknowledgments

This project benefited from information and advice from many individuals. Any errors or misstatements are due to the authors, not the many contributors.

The authors gratefully acknowledge Alwynelle Ahl (USDA-ORACBA), Nathan E. Bauer (USDA-FSIS), Paul Brown (NIH), Linda Detwiler (USDA-APHIS), David W. Harlan (Taylor Byproducts, Inc), and Pedro Piccardo (Indiana University) for their scientific input and critical review of different sections of the manuscript. We also thank Drs. H. Christopher Frey, John C. Galland, Junyu Zheng, Bram E.C. Schreuder, and John Wilesmith who reviewed the original report (November, 2001) for the USDA.

In addition we would like to thank the following individuals for providing scientific input and support:

Gary Adams, Texas A&M University
John Adams, National Milk Producers Federation
Judd Aiken, University of Wisconsin-Madison
Steve Anderson, USDA-FSIS
Haluk Anil, Bristol University
David Asher, FDA
Harry Baker, Cambridge University
Mike Barry, Oklahoma City Zoo
Daniel Bohnenblust, Swiss Federal Statistical Office
Robin Bolin, ConAgra Beef Company
David Bolton, New York State Institute for Basic Research
Robert Brady, USDA-APHIS
Gerald R. Bratton, Texas A&M University
Robert Brewer, USDA-FSIS
Victoria Bridges, USDA-APHIS
Moira Bruce, Institute for Animal Health, Neuropathogenesis Unit, Edinburgh
Herbert Budka, University of Vienna
Terry Caviness, Caviness Packing Company
Richard Cawthorrne, Ministry of Agriculture, Fisheries and Food, UK
Megna Chakrabarti, Harvard Center for Risk Analysis
Nguyen Chau, Harvard Center for Risk Analysis
Nick Chesnut, Union County Feedlot, New Mexico
Lily Chounlamountry, USDA-FSIS
John Clay, Dairy Records
Michelle Colby, Virginia-Maryland Regional College of Veterinary Medicine
Phillip Comer, DNV
Nancy Cook, Pet Food Institute
Maria Correa, North Carolina State University
Barbara Corso, USDA-APHIS
Ken Crandall, DHI-Provo
Randall Cutlip, USDA-Craig White, USDA-FSIS
Dyan Daly, National Food Center, Dublin
Dave Dargatz, USDA-APHIS
Arthur Davis, USDA, NVSL
Bryan Dierlam, American Cattlemen's Association

Marcus Doherr, BVET, Switzerland
Christl Donnelly, Oxford University
Basil Eastwood, USDA-CSREES
Dan Englejohn, USDA-FSIS
Lisa Ferguson, USDA-APHIS
Neil Ferguson, Oxford University
J. Fetrow, University of Minnesota
Lloyd Floyd, Kansas Dept. of Wildlife & Parks
Don Franco, National Renderers Association
Azra Ghani, Oxford University
Randy Gordon, National Grain & Feed Association
Daniel Gould, Colorado State University
George Graber, FDA
Temple Grandin, University of Illiniois
John Gray, USDA-APHIS
Dicky Griffin**,** University of Nebraska
Scott Hafner, USDA-APHIS
Mark Hall, USDA-APHIS-NVLS
Rachael Hamilton, American Meat Science Association
J Hamir, USDA-ARS-NADC
Jeff Hansen, Murphy Farm Inc
Michael Hansen, Consumer's Union
David Harbour, Bristol University
Dagmar Heim, BVET, Office Veterinaire Federal, Switzerland
Jud Heinrichs, Penn State University
Christopher Helps, Bristol University
Peter Hewson, Ministry of Agriculture, Fisheries and Food, UK
Harold Hodges, BFD Corporation
Paul Honeyman, Ministry of Agriculture, Fisheries and Food, UK
John Honstead, FDA
Will Hueston, Virginia-Maryland Regional College of Veterinary Medicine
Nora Hunter, Institute for Animal Health, Neuropathogenesis Unit, Edinburgh
James Ironside, CJD Surveillance Unit, Edinburgh
Alan Jenny, USDA-APHIS, NVLS
Sherri Kochevar, ConAgra Beef Company
Chuck Lambert, American Cattlemen's Beef Association
John Lawrance, University of Iowa Extension Service
T. Lindley, TAMA
Ernest Justus Lücker, -Liebig University
Terry Lutz, USDA-AMS
Donna Malloy, USDA-APHIS
Jeronimo Mamaclay, USDA-FSIS
Jeffrey Martin, VLA Lasswade, Scotland
Danny Matthews, Ministry of Agriculture, Fisheries and Food, UK
Patrick McCaskey, USDA-FSIS
Dan McChesney, FDA
Janice Miller, USDA-ARS, NADC
Michael Miller, Colorado Division of Wildlife
Ray Millers, BFD Corporation
Burt Mitchel, FDA
Sarah Muirhead, Feedstuff Magazine

Dennis K. Mullane, Taylor By-Products, Inc
H.D. Norman, USDA-ARS
Greg Oliver, Australian Quarantine and Inspection Service
Arnold Paft, USDA-NAH
George Patrick, Taylor Packing Co. Inc
Alan Pernaa, United Cooperative Farmers Inc
Jody Pinter, AgSource Cooperative Services
Teresa Platt, Fur Commission USA
Ilpo Pölönen, Finnish Fur Breeders' Association
Burt Prichett, FDA
Jim Quigley, Animal Protein Corporation, Inc
Rosalind Ridley, Cambridge University
Elisa Rubi, Direccion General de Salud Animal, Mexico
R. Rubinstein, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY
Larry Satter, USDA-ARS
Mary Jo Schmerr, USDA-ARS-NADC
Glen Schmidt, Colorado State University
Chuck Schuad, University of New Hampshire
Richard Sellers, American Feed Industry Association
Craig Shultz, USDA-FSIS
Bill Smith, USDA-APHIS
L. Smith, National Nutrition Program Advisor, USDA
Charlie Sniffen, Miner Institute
Walt Solonynka, Conagra Beef Company
Robert Somerville, Institute for Animal Health, Neuropathogenesis Unit, Edinburgh
John Spouge, DNV
Richard Theise, International Natural Casing Association
David Thomas, Extension Sheep Specialist, Madison, WI
Judy Thompson, Canadian Food Inspection Agency
Chris Toyne, Agriculture and Fisheries Branch, ABARE, Australia.
Donald Upton, United Cooperative Farmers Inc
Andrea Vicari, North Carolina State University
James Voss, Colorado State University
Paul Walker, Illinois State University
Gary Weber, National Cattlemen’s Beef Association
Gerald Wells, Central Veterinary Laboratory, UK
Marion Weybridge, Ministry of Agriculture, Fisheries and Food, UK
Craig White, USDA-FSIS
John Wilesmith, Central Veterinary Laboratory, UK
Jim Wilkus, USDA-FSIS
Robert Will, CJD Surveillance Unit, Edinburgh
Elizabeth Williams, University of Wyoming

Table of Contents

Executive Summary

In 1998 the United States Department of Agriculture asked the Harvard Center for Risk Analysis to evaluate the robustness of U.S. measures to prevent the spread of bovine spongiform encephalopathy (BSE or "mad cow disease") to animals and humans if it were to arise in this country. BSE is a member of a family of diseases that includes scrapie in sheep and goats, chronic wasting disease in certain North American deer and elk, transmissible mink encephalopathy, and the human ailments Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, and Kuru.

We have developed a probabilistic simulation model to help characterize the consequences of introducing BSE into the U.S. *via* various means. Our model allows us to predict, for example, the number of newly infected animals that would result from introduction of BSE, the time course of the disease following its introduction, and the potential for human exposure to infectious tissues. We evaluate key processes and procedures that make the spread of disease more or less likely. Results are presented as distributions reflecting the probabilistic nature of the model and the processes simulated.

Our analysis finds that the U.S. is highly resistant to any introduction of BSE or a similar disease. BSE is extremely unlikely to become established in the U.S. For example, in a hypothetical scenario in which ten cattle infected with BSE are imported into the U.S., on average only four new cases of BSE would occur. Moreover, the disease is virtually certain to be eliminated from the country within 20 years after its introduction. These results assume that the conditions affecting the spread of BSE in the U.S. would remain unchanged for the 20 years following its introduction. The new cases of BSE would result primarily from lack of compliance with the regulations enacted to protect animal feed. The import of one infected animal yields on average less than one new BSE case in 20 years and the disease is likely to be quickly eliminated from the U.S. following its introduction. Likewise, there appears to be no potential for an epidemic of BSE resulting from scrapie, chronic wasting disease, or other cross-species transmission of similar diseases found in the U.S. Even if they existed, these hypothetical sources of BSE could give rise to only around two cases per year. Similarly, if the disease does indeed occur spontaneously in cattle, as some have suggested, it would result in one to two cases per year with little spread.

Only a small amount of potentially BSE-contaminated tissues would reach the human food supply and be available for possible human consumption. We express the amount of infectivity in terms of cattle oral ID_{50}s for the purpose of quantifying both animal and human exposure to this agent. A cattle oral ID_{50} is the amount of infectious tissue that would, on average, cause 50% of exposed cattle to develop BSE. The relationship between human exposure quantified in terms of cattle oral ID_{50}s and likelihood of human disease is unknown, but European authorities suggest that the cattle disease may be 10 to 100,000 times less virulent in humans (European Union Scientific Steering Committee 1999a; European Union Scientific Steering Committee 2000a). In the entire 20 year period following the import of ten BSE-infected cattle, the mean estimate for the amount of infectivity potentially available for human exposure is 39 cattle oral ID_{50}s. The greatest sources of infectivity include consumption of cattle brain, spinal cord, and meat derived from advanced meat recovery systems. Some potential exposure would result from the presence of spinal cord in certain bone-in cuts of beef, like T-bone steaks, and consumption of cattle intestines. Potential human exposure resulting from spontaneous disease or cross-species transmission of scrapie is predicted on average to be no more than 100 cattle oral ID_{50}s over 20 years.

Even in an extreme case, which we characterize using the 95^{th} percentile of the output distribution from the simulation, the import of ten infected animals leads to only 16 new cases of BSE over twenty years. The 95^{th} percentile value for potential human exposure is 180 cattle oral ID_{50}s over 20 years, nearly five times the mean value. These predictions can be compared with the experience in the United Kingdom, where it is estimated that there were nearly one million infected animals and it is likely millions of cattle oral ID_{50s} available for potential human exposure.

Measures in the U.S. that are most effective at reducing the spread of BSE include the ban on the import of live ruminants and ruminant meat and bone meal from the UK (since 1989) and all of Europe (since 1997) by USDA/APHIS, and the feed ban instituted by the Food and Drug Administration (FDA) in 1997 to prevent recycling of potentially infectious cattle tissues. This feed ban greatly reduces the chance that BSE will spread from an infected animal back to other cattle through feed. Our model reflects incomplete compliance with the FDA feed ban and we evaluate the potential risks resulting from exceptions to the ban. Measures instituted in meat packing plants by the industry and the USDA Food Safety Inspection Service (FSIS) have reduced the opportunity for infectious tissues to contaminate human food.

Specific pathways or practices that would contribute the most to the spread of BSE if it were introduced into the U.S. relate to compliance with the FDA feed ban and include misfeeding on the farm (*i.e.*, administration to cattle of feed labeled as prohibited for that purpose) and the mislabeling of feed and feed products prohibited for consumption by cattle. The disposition of cattle that die on the farm would also have a substantial influence on the spread of BSE if this disease were introduced into the U.S. Factors that influence potential human exposure include the handling of brain and spinal cord in processing plants and how well inspectors would detect animals with BSE at slaughter.

Our model is not amenable to formal validation because there have been no controlled experiments in which the consequences of introducing BSE into a country has been monitored and measured. However, as a test of the model's plausibility, we modeled the small BSE outbreak identified in Switzerland following the introduction of BSE infectivity from the UK. Working with experts in Switzerland, we identified appropriate values for model parameters necessary to appropriately characterize that country's practices and procedures and then simulated the introduction of BSE infectivity. Our simulation took into account risk management actions, such as feed bans instituted by the Swiss. The model's predictions were reasonably close to empirical observations. For example, the model predicted that during the Swiss outbreak, there would have been 230 animals that developed clinical signs of disease. To date, the Swiss have detected 398 animals with BSE. The time course of the outbreak predicted by the model also reasonably resembled the pattern observed in Switzerland. The ability of the model to reasonably replicate the magnitude and time course of the Swiss outbreak gives some confidence in the structure of our model, especially in light of the many unknown factors associated with this episode. In fact, modest revision of highly uncertain assumptions from our "first guess" values, based on advice from personnel from the Swiss Federal Veterinary Office, yielded results that were even more consistent with observed data.

We also evaluated the potential for BSE to have entered the U.S. prior to the 1989 ban on the import of UK cattle. BSE has not been detected in the U.S. despite 12 years of active surveillance of high-risk animals. Yet several groups, including the European Union in their Geographically Based Risk Assessment of the U.S. (European Union Scientific Steering Committee 2000d), have highlighted the 334 animals brought into the U.S. from the UK between 1980 and 1989. These animals were imported as breeding stock, not as beef or dairy production

animals. This fact is likely to have reduced their potential for exposure to BSE before their export from the UK. In addition, none of these animals came from a farm on which there was a case of BSE in animals from the same birth cohort (same birth farm and year). Many came into the U.S. before BSE was even a recognized disease (the first case was confirmed in the UK in 1986). The USDA has identified and traced the disposition of these animals and has verified that 161 were disposed of in a manner that poses no risk to other animals or to humans. However, the Department has not been able to conclusively make this determination for the remaining 173 animals. Using data identifying the year of birth, the year of import, the date of the animal's last known sighting, and information characterizing the time course of the disease following infection, we have estimated the amount of BSE infectivity that could have theoretically been introduced into the U.S. from these 173 animals. We then used this estimate in our model to predict the possible consequences in the U.S.

Our analysis concludes that there is more than an 80% chance that the import of these animals resulted in no exposure of U.S. cattle to BSE infectivity. Even if U.S. animals were exposed to BSE, there is a significant chance that the exposure resulted in no new cases of disease. Our analysis indicates that there is only a small chance that BSE spread to U.S. cattle but that the number of cases was sufficiently small to avoid detection by U.S. government surveillance. The analysis also shows that if these imports did introduce BSE into the U.S., measures taken by the government and industry since 1997 will have arrested the disease and begun to eradicate it.

Our evaluation of potential risk mitigation actions highlights potential measures to further reduce the already low likelihood that if BSE were introduced into the U.S., it could spread to cattle or contaminate human food. Prohibiting the rendering of animals that die, potentially from BSE, prior to being sent to slaughter (*i.e.*, animals that "die on the farm") substantially reduces the potential for contamination of cattle feed, decreasing the average predicted additional cases of BSE following introduction of ten infected cattle by more than 80%. Implementation of a UK-style ban on specified risk material (*e.g.*, spinal cords, brains, vertebral columns) from both human food and animal feed reduces the predicted number of additional BSE cases in cattle by almost 90% and potential human exposure by 95%. These findings serve to illustrate the types of evaluations of alternative risk management strategies that can be conducted using the model.

In summary, measures taken by the U.S. government and industry make the U.S. robust against the spread of BSE to animals or humans should it be introduced into this country. Preventing infected animals or contaminated feed from entering the country, ensuring compliance with the FDA feed ban, and reducing the potential for contaminated tissues to enter the animal feed or human food supply will ensure that these risks remain low. If BSE has been introduced into the U.S., as has been suggested by some observers, the course of the disease has been arrested and it is destined for eradication by the measures currently in place.

1 Introduction

Bovine spongiform encephalopathy (BSE) is a disease of cattle that was first documented in the United Kingdom in 1986. It has since spread to several countries in Europe, and most recently to Japan. The disease causes the degeneration of central nervous system (CNS) function, ultimately leading to death in all cases. Perhaps more worrisome is the possibility that meat products contaminated with BSE infectivity[1] can cause a human form of this illness, known as variant Creutzfeldt-Jakob Disease, or vCJD. Like BSE, vCJD causes CNS degeneration and is always fatal. Unlike many other animal-borne diseases, the agent thought to be responsible for BSE and possibly vCJD is at least partially resistant to destruction by standard cooking practices, sterilization procedures, and processes used to recycle bovine protein prior to its use as a feed supplement. For that reason, the presence of BSE can lead to the spread of disease among other animals, and potential health risks for people.

Although there has never been a case of BSE documented in the United States, the potential for the disease to spread, and the potential threat it poses to people has raised concern in this country. In order to better characterize the nature of these risks, the United States Department of Agriculture (USDA) commissioned the Harvard Center for Risk Analysis to conduct a study of BSE in the U.S.

This study was undertaken to investigate potential pathways by which BSE or other TSEs could arise in the United States (U.S.) cattle population[2]. In particular, the analysis describes the use of a quantitative simulation model that characterizes how the introduction of BSE would affect animal health over time, and the extent to which it could result in human exposure to contaminated food products. The ability of this model to quantify various aspects of the disease's progression (*e.g.*, number of animals infected over time, quantity of the transmissible agent in food presented for human consumption) distinguishes it from other efforts to characterize BSE risk, such as the European Union's Scientific Steering Committee report on the Geographical Risk of Bovine Spongiform Encephalopathy (European Union Scientific Steering Committee

[1] Although the exact etiology of BSE is uncertain, in many respects the transmission of the disease can be evaluated as though it arises from an infectious agent. Because the nature of the agent is still a matter of some scientific debate, we use the term "infectivity" to characterize materials that can transmit the disease from one animal to another or potentially from animals to people.

[2] For the purpose of the study, "other TSEs" are defined to be naturally occurring animal prion diseases that if present in cattle, will manifest with clinical and histopathological characteristics that are similar to those

2000c). We have used the simulation model to determine the impact of possible past introductions of BSE into the U.S., to identify those risk management control options that most influence the spread of disease, and to identify those sources of uncertainty that have the greatest impact on our results. This information can be used to help identify the most promising control measures and to prioritize data collection and research efforts.

A key goal of this analysis is to determine whether BSE would develop into a self-sustaining epidemic if it were introduced into the U.S., or if its prevalence would tend to decrease over time, leading eventually to its eradication. Which of these two possibilities occurs depends on the average number of new cases of disease that result from each existing case. This value, designated R_0, is referred to as the epidemic's basic reproduction rate (Anderson 1991). If R_0 exceeds unity, the disease will tend to spread. On the other hand, if R_0 is less than unity, the number of cases will tend to decline over time, and ultimately the disease will die out. Assuming that an initial introduction of BSE into the U.S. is limited in magnitude, the ultimate impact of the introduction will depend vitally on whether R_0 exceeds unity. This concept corresponds to the issue of "stability" that is a focus of the European Union's approach to geographic BSE assessments (European Union Scientific Steering Committee 2000c; European Union Scientific Steering Committee 2000d).

The analysis is not a complete human health risk assessment in two respects. First, we do not quantify the probability that BSE will be introduced into the U.S. Hence, all our risk estimates are conditional on hypothetical scenarios. Second, although we quantify potential human exposure to BSE-contaminated food products, we do not estimate how many people will contract variant Creutzfeldt-Jakob Disease (vCJD). We have omitted quantitative treatment of both of these issues because the available information is inadequate.

The remainder of this study is organized as follows. Section 2 first describes the different types of TSEs, their characteristics, theories as to the origin of the BSE epidemic in the UK, and measures taken to control the spread of BSE. Next, Section 2 discusses the pathways for the introduction of disease in further detail, including the potential for spontaneous development of BSE, transmission from another species in the U.S. with a prion disease, the import of BSE-infected cattle, or use of cattle rations that may contain contaminated material. Section 3

---

associated with cattle BSE. For the remainder of this document, the term "BSE" will collectively refer to BSE and to these other TSEs.

describes our methodology, including the simulation model used in this analysis and the specific scenarios evaluated. Section 4 summarizes our results, and concludes with a discussion of our findings.

2 Background

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

2.1 Overview of Transmissible Spongiform Encephalopathies (TSEs)

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

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

TSEs have also been observed in exotic cats and ungulates in zoo collections. The source of infection is not known. Other examples, such as Feline Spongiform Encephalopathy (FSE), are thought to be the result of cross-species transmission of a TSE (BSE in the case of FSE). Variant CJD (vCJD) is a newly discovered TSE of humans and is likely the result of exposure to the BSE agent.

The first records of TSEs date back to the early 18th century (Stockman 1913; Brown 1998b), with the mention of scrapie in sheep. The name of the disease reflects its associated clinical signs, including the tendency of the sheep to scrape off their wool on fences or other objects. Experimental transmission of scrapie to other species, such as mice and goats, demonstrated that the disease was transmissible and had a very long incubation period (Cullie 1936; Cullie 1939; Pattison 1959).

During the 1950s, many scientists became interested in Kuru, a fatal disease that affected the Fore population of Papua, New Guinea. Kuru is characterized by neurologic signs and neuropathologic changes similar to those of scrapie although it has other signs (e.g., myoclonus) not seen in scrapie but found in other TSEs (Klatzo 1957; Zigas 1957; Alpers 1970). These similarities were pointed out by Hadlow in 1959, who suggested that Kuru also might be transmissible to other animals (Hadlow 1959). Subsequently, Gadujsek and his colleagues succeeded in transmitting Kuru to chimpanzees (Gajdusek 1966). This experiment supported the hypothesis that Kuru is transmitted by an infectious mechanism (*i.e.*, ritualistic cannibalism). Later, other spongiform encephalopathies were found to be transmissible, including CJD, FFI, TME, CWD, and BSE. To date, most of the experimental data on TSEs comes from studies of CJD and scrapie. More recently, BSE has become an area of active research.

The specific agent responsible for TSE diseases has not been identified with certainty, but the leading theory suggests that the etiologic agent is an abnormally configured protein normally encoded by the host (prion protein or PrP) (Bolton 1982; Prusiner 1982; Prusiner 1994; Prusiner 1998). Normal prion protein (PrP^{c}) is soluble in detergents and has a predominantly α-helical structure. In contrast, abnormal PrP (PrP^{sc}) is insoluble in detergents, relatively resistant to proteases, and has a predominantly β-sheet secondary structure. Although still a matter of controversy, PrP^{sc} appears to accumulate in an infected host and eventually cause disease (Hsiao 1991; Bueler 1993; Telling 1995; Manson 1999; Hill 2000). Deposits of PrP^{sc} in tissues are associated with the presence of transmissible infectivity (McKinley 1983). Additionally, PrP^{sc} is the only molecular marker specific for TSE infections. Spongiform degeneration, neuronal vacuolation, and gliosis appear to be associated with abnormal PrP deposition. Remarkably, TSE infection has been reported in the absence of detectable PrP^{sc} (Lasmezas 1997) and PrP^{sc} formed *in vitro*, by conversion of PrP^{c}, has not yet produced disease in animal bioassays (Hill 1999). The etiologic agent is not inactivated by treatments that usually destroy bacteria and viruses

(Kimberlin 1983; Taylor 1991a; Taylor 1991b; Taylor 1993). No immune response to the agent has been detected.

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

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

The remainder of Section 2.1 has three parts. Section 2.1.1 discusses the means by which TSEs are passed from one animal to another, and perhaps from animals to humans. Section 2.1.2 introduces the concept of the "species barrier," a phenomenon that makes passage of a TSE from one species to another far less "efficient" (and hence less likely) than passage between animals of the same species. Finally, Section 2.1.3 discusses susceptibility, *i.e.*, the tendency for some animals to be more likely than others to become infected following exposure to the infective agent.

2.1.1 Transmissibility

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

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

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

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

intra-peritoneal (i.p.) administration of infectivity is estimated to be 100 times less efficient than i.c. transmission (Kimberlin 1988).

2.1.2 The Species Barrier

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

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

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

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

highly replicable differences in the incubation period, neuropathology, and host range (Fraser 1968; Bruce 1989). CJD, scrapie, TME, and CWD show strain diversity, while BSE appears to be a single, stable strain (Bruce 1994; Bruce 1997). vCJD (a disease in humans thought to result from exposure to the BSE agent) does not demonstrate morphologic strains (Will 1996; Bruce 1997; Hill 1997; Scott 1999).

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

Because the presence of a TSE agent is often assessed by inoculating a test species (*e.g.*, mice) with the suspect material, the species barrier reduces the sensitivity of these bioassays. Cattle-to-cattle transmission of BSE by the intracerebral route is estimated to be at least 1,000 times more efficient than cattle-to-mouse transmission by the same route (Section II.7 in (European Union Scientific Steering Committee 2000a)). It is often assumed that the species barrier decreases transmission efficiency by a factor of between 1 (no decrease) and 1,000 (Det Norske Veritas 1997). The assumption that the species barrier is 1 (*i.e.*, that there is effectively no species barrier) is considered to be a worst-case scenario. In an opinion on the species barrier for transmission of BSE from cattle to humans, the EU Scientific Steering Committee (1999a) suggested that plausible values for the species barrier range from 1 to 100,000. For risk assessments, this range was later updated to include 1 as a worst case and a values from 10 to 10,000 as more likely (p. 35 in (European Union Scientific Steering Committee 2000a)). However, the committee concluded that it is not at this time possible to quantify the species barrier for transmission of BSE from cattle to humans (p. 34 in (European Union Scientific Steering Committee 2000a)).

2.1.3 Susceptibility

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

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

An important physiologic factor that is likely to affect susceptibility to infection is the age of the animal. For example, young cattle are estimated to be ten times more susceptible than adults, with data well described by a model that assumes susceptibility declines exponentially with an annual rate constant of 0.85 after the age of four months, and susceptibility ultimately declining to 10% of its peak value (de Koeijer 1999). Wilesmith *et al.* (1988) described an epidemiologic study of the UK BSE epidemic. Based on a model of that event, Anderson *et al.* (1996) back-calculated an alternative susceptibility estimate suggesting that susceptibility peaks at 1.31 years and decreases in the following years. Other investigators estimated that susceptibility in cattle peaks at between 0.5 to 1.5 years of age (Woolhouse 1997). Age-related susceptibility is hypothesized to be associated with permeability of the intestine to large proteins and with the development of the Peyer's Patches (PP). The PP seem to play a role in the pathogenesis of the prion diseases and to influence the susceptibility of the animal to infection. For example, in sheep, the ileal PP are shown to be more active and to be largest when the animal is around 2-3 months old and to disappear when the animal reaches an age of one and one-half years (Griebel 1996). The appearance of the PP in sheep appears to coincide with the period of greatest susceptibility of sheep to scrapie (Hadlow 1982; Andreoletti 2000).

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

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

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

Sheep

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

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

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

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

Humans

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

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

Bovines

Investigators have identified polymorphisms in the PrP gene in British cattle, Belgian cattle, and US cattle (for a review refer to (European Union Scientific Steering Committee 2000a)). There are two major polymorphisms in the region of the PrP gene: 1) the HindII

restriction site, and 2) differences in the number of copies (five or six) of an octapeptide repeat sequence (Goldmann 1991; Hunter 1994).

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

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

Cattle have been shown to be partially susceptible to naturally induced scrapie but only following intracerebral injection of infectious material (Gibbs 1990; Cutlip 1994; Clark 1995; Cutlip 1997; Cutlip 2001). Studies done in the U.S. showed that cattle orally exposed to North American scrapie remain normal for eight years following exposure (Cutlip 2001). Research on cattle orally exposed to UK scrapie is ongoing (Detwiler 2000). Currently, there are no available data indicating how genetics might influence bovine susceptibility to scrapie.

Cervids

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

2.2 The Origin of the BSE Epidemic in the UK

In 1986, a bovine spongiform encephalopathy was first confirmed in the United Kingdom as the result of routine animal disease surveillance. This section focuses on theories advanced to explain the origins of the subsequent epidemic. Since the beginning of the epidemic, over 178,400 cases have been confirmed on 35,275 farms. In addition, cases have been observed in Northern Ireland, the Republic of Ireland, and in other European countries (OIE 2000). Mathematical modeling suggests that the epidemic probably started in the UK between 1981 and 1982 (Wilesmith 1991; Wilesmith 1992b; Wilesmith 1994; Donnelly 1997b; Ferguson 1997b; Ferguson 1999). The epidemic peaked at the end of 1992-1993 when the incidence reached approximately 3,500 confirmed cases per month. Although the origin of the BSE epidemic remains controversial, there is little doubt that it was maintained by the recycling of bovine materials in the bovine feed chain (Wilesmith 1991; Wilesmith 1992b; Kimberlin 1994; Wilesmith 1994; Nathanson 1997).

Although the effectiveness of the feed ban and other measures at reducing the incidence of BSE in the UK sheds light on the progress and amplification of this epidemic (Section 2.4.2), its precise origin remains uncertain (Wilesmith 1991; Wilesmith 1992a; Wilesmith 1993; Hoinville 1994; Brown 1998a). The most prominent theory hypothesizes that BSE occurred when the scrapie agent, present in rendered proteins used in feed, overcame the species barrier to infect cattle (Section 2.2.1). Several changes in rendering and feeding practices may have enabled the infectious agent to survive during rendering process and enter the cattle feed chain (Taylor 1989; Horn 2001). An alternative theory postulates a spontaneous case of BSE as the origin (Section 2.2.2). Section 2.3.1 discusses spontaneous disease in further detail. Additional theories focus on different infectious organisms or toxic agents that could cause a spongiform encephalopathy or on dietary imbalances known to produce spongiform encephalopathies under some conditions (Section 2.2.3).

2.2.1 Scrapie in Sheep

Evaluating the hypothesis that scrapie is responsible for the BSE epidemic in the UK is complicated because even transmission of this disease among sheep is not well understood. Horizontal transmission may involve the shedding of the agent into the environment (Hoinville 1996; Stringer 1998; Woolhouse 1998). Maternal transmission from ewe to lamb in utero or

immediately during the post-natal period is believed to occur, although there has been some debate in the past (Ridley 1996). Maternal transmission of scrapie may explain why the disease usually becomes endemic in a flock once it is introduced. Exposure through contaminated vaccines (Gordon 1939; Gordon 1946; Gordon 1959; Agrimi 1999; Caramelli 2001) has been documented as a source of infection. Transmission of scrapie *via* vectors is disputed (Fitzsimmons 1968; Hourrigan 1979).

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

One finding supporting this theory is the observation that BSE apparently originated at several locations at nearly the same time (Kimberlin 1994; Wilesmith 1994; Nathanson 1997). Such a pattern suggests some sort of population-wide insult, such as a large supply of ineffectively treated feed containing scrapie. This theory also is supported by the finding that cattle are susceptible to infection by scrapie introduced by i.c. experimental inoculation. On the other hand, investigators have been unable to infect cattle with the North American scrapie agent when it has been orally introduced (Cutlip 1994; Cutlip 1997; Cutlip 2001); research is ongoing using the UK scrapie agent (Detwiler 2000). In addition, if scrapie did cause the development of BSE in cattle, it is not clear why it happened suddenly in 1986 at several locations given that scrapie has been endemic in European sheep for over 250 years and ovine and bovine wastes have been used in cattle feed for several decades. In response to that question, attention has focused on the use of MBM in dairy calf starter rations, feed given to very young animals (Horn 2001). This

practice, which is not likely to have taken place elsewhere (except in Australia, which is a scrapie-free country) would have exposed animals to infectivity when they are most susceptible. Finally, if scrapie caused BSE to originate at several locations at nearly the same time, it is surprising that there is only a single strain of BSE even though sheep in the UK are known to carry several "strains" of scrapie.

One variation on the scrapie hypothesis suggests the existence of a strain of scrapie that was more thermostable and particularly infectious to cattle. This theory suggests that this strain may have entered the cattle feed chain as a component of MBM. The specific strain may have been a mutation of the scrapie agent. It is possible that either: 1) a single scrapie strain with characteristics unlike BSE was transmitted to cattle and that these characteristics changed as the agent was repeatedly recycled through cattle; or 2) a BSE strain pre-existed in sheep, and was unchanged when passed to cattle. These hypotheses are both plausible. However, if the origin of BSE was a single "strain" of scrapie, the BSE epidemic should have had a more geographically compact origin than the diffuse pattern actually observed, unless the single "strain" was widely distributed. BSE epidemiology shows a geographically widespread occurrence with simultaneous onset at multiple distant locations, rather than an origin focused at a single point. Finally, this alternative theory does not address the lack of a demonstration to date of *any* strain of scrapie that can infect cattle following oral administration (Cutlip 2001).

2.2.2 Infrequent Sporadic BSE

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

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

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

2.2.3 Toxic Agents and Other Hypotheses

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

2.2.3.1 Organophosphate (OP) Pesticides

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

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

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

2.2.3.2 Copper Deficiency

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

One theory for the origin of the BSE epidemic hypothesizes that the high levels of protein in feed used in the 1970s and 1980s competed with copper for absorption by ruminants (Rehbinder 1994). This theory is consistent with some aspects of the disease, but the morphology and distribution of vacuolar or spongiform-like changes observed in animals suffering from copper deficiency differ from the spongiform changes that are typical of a TSE (Annex 6 in (European Union Scientific Steering Committee 2000b)). This theory also fails to account for the disease's transmissibility. Furthermore, if this hypothesis were valid, beef cows (that obtain most of their nutrients through pasture) should have a higher incidence of BSE than dairy cattle, and the reverse is observed (Horn 2001).

2.2.3.3 Heavy Metal Exposure

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

2.2.3.4 Autoimmune Disease

Investigators have proposed that BSE could be an autoimmune disease caused by exposure of cattle to bacteria containing proteins that induce immunologic cross-reactivity with central nervous system tissue (Ebringer 1997; Tiwana 1999). According to this theory, the BSE epidemic resulted from the production of feed rich in the specific bacteria with proteins that mimic brain tissue. Because of exposure to these bacteria, bovine anti-bacterial antibodies may have reacted with myelin proteins in the brain that were sufficiently similar to bacterial antigens. The damage caused by such an auto-immune disease would be chronic and would be consistent with some of the characteristics of BSE (European Union Scientific Steering Committee 2000b). Autoimmune brain disease can be experimentally transmitted to animals but only by injecting large amounts of brain protein and adjuvants. In contrast, TSE can be transmitted by injecting only a few nanograms of brain without adjuvant (Horn 2001). In addition, the neuropathology of autoimmune brain disease differs from that observed in TSE.

SSC has concluded that this theory is not consistent with the BSE epidemic for several reasons. First, the morphology and distribution of vacuolar or spongiform-like changes due to BSE are not the same as those caused by an auto-immune reaction. Second, lymphocytic infiltrates that are typical of and are a pathogenetically important component of auto-immune disease are atypical of TSE. Third, high infectious titers cannot be explained by the autoimmune hypothesis (European Union Scientific Steering Committee 2000b). Fourth, as pointed out by the BSE Inquiry (Lord Phillips 2000), mouse-adapted BSE can be transmitted i.c. to mice lacking a functional immune system. Fifth, common bacteria do not have the resistance to chemical and physical inactivation shown by the agents of transmissible spongiform encephalopathies, including bovine spongiform encephalopathy. Finally, this theory fails to explain why many thousands of animals suddenly became affected and why the BSE epidemic occurred predominantly in the UK (Horn 2001).

2.2.3.5 Use of Pituitary Hormones

Posterior pituitary extracts obtained from oxen or other mammals were used as a source of oxytocin for veterinary purposes through the period 1950-1989. Use of anterior pituitary extracts as a source of follicle stimulating hormone or bovine growth hormone occurred but was not widespread (Horn 2001). Early epidemiologic investigations of the BSE outbreak dismissed hormone extracts as a cause of the disease's spread (Wilesmith 1988). Recall that growth

hormone, prepared from cadaver pituitary glands, has caused transmission of CJD in humans, and this theory suggests that a similar phenomenon may have occurred in cattle (Airtime 2001). This hypothesis suggests that pituitary hormones contaminated by BSE caused the epidemic in the UK but not in other countries. The source of the original infectivity (in humans it was one or more pituitaries from CJD cases) is not explained by this theory.

2.2.3.6 Wild African Antelope

A group of scientists from New Zealand postulated that BSE originated from wild African antelope, and that it spread into British cattle when an infected animal from a wildlife park was rendered into MBM that was fed to about 1,000 dairy cows in the southwest of England between 1975 and 1977 (ProMED-mail 2001, April 18). They hypothesized that the disease probably spread through British cattle for about a decade before it was fully recognized. Kelly et al. (1980) suggested that the death of six white tigers in a British zoo was due to TSE but the histopathology was not consistent with BSE (Horn 2001). This theory is not consistent with the absence of evidence that carnivores in zoos in the UK were dying from TSE-like symptoms before 1986. In addition, there is no evidence that any TSE exists in the wildlife of Africa (Horn 2001).

2.3 Sources of BSE Infectivity

This section describes potential sources and pathways by which BSE infectivity could be introduced into the U.S., including the development of a spontaneous BSE case (Section 2.3.1), the import of an infected animal into the U.S. (Section 2.3.2), scrapie (Section 2.3.3), oral ingestion of tissue or material containing chronic wasting disease infectivity (Section 2.3.4), horizontal or lateral transmission of chronic wasting disease (Section 2.3.5), transmissible mink encephalopathy (Section 2.3.6), TSEs in pigs (Section 2.3.7), TSEs in chickens (Section 2.3.8), and contamination from recycled products, including plate waste, gelatin, milk, blood and blood products, and tallow (Section 2.3.9).

2.3.1 Spontaneous BSE

A potential way in which BSE could be introduced into the United States is the development of a spontaneous case of a BSE in a native animal. A “spontaneous case” is one that occurs in an animal with no known risk factors for development of BSE. The presumed

mechanism by which a BSE could occur spontaneously is by the mutation of the PrP gene to a form that codes for PrP^{sc}, and subsequent recruitment of PrP^{c} until disease is manifest (Prusiner 1989); (for a review see: (Chesebro 1999). There is no direct evidence of this mechanism, although some argue that all mammals might have a low spontaneous rate of TSE (Hueston 1997). In addition, a transgenic animal over-expressing the PrP gene has apparently replicated the human TSE GSS (Hsiao 1991). Recent results, in which mice expressing the same point mutation but at normal levels failed to develop disease (Manson 1999), suggest the mutations may increase susceptibility rather than directly cause the disease. Although at this time there is no scientific evidence suggesting that spontaneous BSE exists, the BSE Inquiry suggested that TSEs could possibly develop sporadically in other species, as they do in humans (BSE Inquiry 2000a). In contrast, the Review of the origin of BSE (Horn 2001) concluded that although the spontaneous case hypothesis cannot be excluded, there is no evidence supporting the presence of sporadic form prion disease in cattle or sheep.

It is not possible to determine for any particular TSE whether the original cause was a mutation or transmission of disease from another species or from the same species. For example, transmissible mink encephalopathy (TME) has no known origin. There are a number of theories, most of which focus on transmission from another species (Marsh 1991). In the case of BSE, there is little evidence from the epidemiology to suggest that cases arise without some exposure to infectivity (Wilesmith 1991; Kimberlin 1994; Horn 2001). On the other hand, there are a small number of cases for which there are no known risk factors in the UK (MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) 2000) and in Denmark (Tegtmeier 2001).

The existence of a spontaneous form of TSEs in animals is controversial. In humans, cases of CJD in persons with no known risk factors or exposure to the disease occur at an annual incidence of approximately one per million. The incidence appears to be relatively constant around the world, regardless of diet, environment, or other factors that may hypothetically influence disease rates. Cases in individuals with no known risk factors are often referred to as "sporadic CJD." The etiology of sporadic CJD is unknown. Sporadic CJD appears almost exclusively in humans more than 50 years old. Cases appear to occur without a predictable epidemiological pattern (Will 1986; Brown 1994b). Sporadic CJD accounts for 85% of all cases and these cases are characterized by a clinical course that is relatively rapid, although rare variants have shown an extensive duration of clinical illness (Brown 1984). It is sometimes

asserted that the rate of sporadic CJD in humans is likely to be representative of the rate of spontaneous BSE in cattle (Biopharm 1997), although the rate in cattle has never been directly measured and may in fact be zero.

2.3.2 Import of BSE Infectivity into the United States

This section describes the potential for the import of BSE infectivity into the U.S., in the form of either infected live cattle (Section 2.3.2.1) or contaminated feed material (Section 2.3.2.2).

2.3.2.1 Imported Live Cattle from the UK

The U.S. imported animals from the UK during that country's BSE epidemic. Between January 1, 1981, and July 1989, the United States imported 334 cattle from the UK. Ninety-six percent of these animals were beef breeding stock, while the remaining four percent were dairy cattle. In 1989, the U.S. prohibited the import of ruminants from countries affected by BSE.

Of the 334 UK imports, 161 were disposed of in a manner that eliminates the possibility that they could have contaminated either human food or animal feed. The remaining 173 cattle were imported before the peak of the epidemic, and none came from a birth cohort in which a BSE case is known to have developed (Detwiler 2001). Of these 173, 164 (94.8%) were beef breeding animals and nine (5.2 %) were dairy animals. It is possible that remains from some of the 173 cattle imported from the UK between 1980 and 1989 could have ended up in either animal feed, human food, or both. Section 3.4.3 describes our risk assessment of this scenario.

2.3.2.2 Imported Cattle from Continental Europe

Between 1983 and 1987 397 breeding cattle were imported from Switzerland, France, Italy, and Belgium. These animals were beef breeds except for ten dairy animals imported from France in 1984. From 1996 to 1997, there were also 46 animals imported from Belgium, Germany, Austria and Italy. Because the vast majority of the imports from continental Europe occurred before 1988, they pose only a limited risk to the U.S. All animals imported after 1996 have been traced and their movements controlled. There is therefore virtually no risk that these imports introduced BSE into the U.S. cattle population. Finally, two head of cattle imported from Belgium in 1996 are also under quarantine.

2.3.2.3 Imported Cattle from Non-European Countries

It is estimated that since 1986, the U.S. has imported from Canada and Mexico between 750,000 and 2.5 million animals annually. The vast majority of the cattle (at least 80%) are animals for feeding or slaughter. The cattle imported from Canada for immediate slaughter are sent to slaughterhouses in sealed trucks. All identification is collected at the time of slaughter and they are noted as animals of Canadian origin. These imports are extremely unlikely to pose a risk of introducing BSE into the U.S.

2.3.2.4 Imported MBM and Feed

There are no reliable data documenting the type and composition of feed imported into the U.S. between 1980 and 1990. Current APHIS regulations prohibit the import of ruminant meat and edible products produced from animals in regions where BSE has been documented.

USDA reports that no MBM was imported from the UK between 1980 and 1990. Since 1989, MBM has been imported from New Zealand, Canada, Chile, Peru, and Australia. Most MBM came from Canada (an average of 25,000 metric tons per year). Chile exported to the U.S. only 3,000 metric tons of MBM in 1989. Peru exported 15,000 metric tons of MBM to the U.S. in 1989 and 4,000 metric tons in 1990. Panama exported 7,000 metric tons of MBM in 1989 and 4,000 tons in 1990. It is important to note that: 1) the U.S. produces approximately 60% of the rendered materials generated globally (Rudbeck 1999); 2) the U.S. is mainly an exporter of MBM; and 3) shipping MBM to the U.S. from overseas is unlikely to be economically competitive (Franco 2001).

The geographical assessment of BSE in the U.S. (European Union Scientific Steering Committee 2000d) reported the export of mammalian meal and flour from the UK in 1981 (12 tons), 1984 (10 tons), 1985 (2 tons), 1989 (20 tons), and 1997 (37 tons). The U.S. does not have corresponding import statistics for 1989 (USDA-APHIS 2000). It is likely that the materials imported in 1997 included non-mammalian protein because the UK Overseas Trade statistics did not specifically report separate values for MBM, but instead subsumes this material in the category, "*flours and meals of meat and offals, unfit for human consumption, greaves*". In addition, since the adoption of the Commission Decision 93/239/EC on March 27, 1996, it has been illegal to export from the UK meat meal, bone meal, and MBM derived from mammals.

MBM imported prior to the peak of the epidemic in the UK is not thought to pose a substantial BSE risk. Most importantly, MBM is used for pet food and other products and poses little exposure risk to cattle. Finally, although it is possible that MBM from the UK entered the U.S. *via* a third country, there is no evidence that has occurred. In summary, past MBM imports pose little risk of exposing U.S. cattle to BSE.

2.3.3 Domestic Scrapie

The first case of scrapie diagnosed in the U.S. occurred in 1947. Various control and eradication programs have existed since 1952 when the disease became nationally reportable. At that time, when the disease was confirmed in a flock, the flock was quarantined and then depopulated. In 1957, the regulations were amended to include location and subsequent depopulation of the of source flocks. Different surveillance approaches have been instituted since that time (*e.g.*, the bloodline surveillance program and reporting incentives). The Voluntary Scrapie Flock Certification Program, approved in 1997 was designed to monitor flocks and certify the status of the animals enrolled in the program (U.S. Department of Agriculture 2000). This program is still in effect.

The precise prevalence of scrapie in sheep in the U.S. is unknown. It is likely that changes in the national scrapie control and eradication program affected the reporting of potentially infected sheep. Statistics from the period between 1947 and 1992 indicate that during this period, there were a total of 1,117 affected sheep from 657 flocks located in 39 states (Wineland 1998). The number of scrapie-positive flocks increased slightly between 1965 and 1992, probably reflecting changes in reporting protocols and changes in incentives offered to farmers to report affected animals. One hundred sixty-eight rams and 949 ewes were also reported to be scrapie-positive during the study period. Annual mortality due to scrapie in a flock is usually low (three to five percent), although higher mortality rates of up to 50% have been reported (Detwiler 1992). In Great Britain, the prevalence of detectable scrapie infection in the slaughter population was estimated to be 0.11% in 1997/1998. This detectable rate probably corresponds to a true infection prevalence in the same population of up to 11% (Webb 2001). Table 2-1 details the number of sheep condemned with scrapie in the U.S.

Table 2-1
Sheep Condemned With Scrapie in the United States:
Fiscal Year 1998

Animal Category	Number Slaughtered	Number Condemned	Percent Condemned
Mature Sheep	181,615	3,480	1.92
Lambs and Yearlings	3,272,844	3794	0.12
Goats	396,473	1505	0.38

The epidemiology of scrapie in the U.S. has reflected changes in the surveillance of the disease, reporting requirements, and in reporting incentives. The geographic concentration of scrapie-positive flocks in certain states has been attributed to the level of surveillance conducted within those states. No seasonal incidence pattern has been associated with scrapie.

The BSE epidemic in the UK influenced the processing of animal proteins in the United States. In 1989, the National Renders Association (NRA) and the American Protein Producers Industry (APPI) recommended a voluntary ban on the processing of dead sheep. Consequently, the proportion of renderers processing inedible sheep offal also declined (Eastern Research Group 1996). Later, the FDA prohibited the use of all ruminant proteins in ruminant feed (U.S. Food and Drug Administration 1997).

2.3.4 Chronic Wasting Disease: Oral Exposure

Chronic-wasting disease (CWD) is a spongiform encephalopathy recognized for the first time in captive deer at a Colorado research facility in 1967 and later in Rocky Mountain elk at a Wyoming research facility in 1978 (Williams 1980). Additionally, CWD was confirmed in mule deer in 1977 (Williams 1980), and in free-ranging deer and elk in a five county region in Colorado and Southeastern Wyoming (Williams 1982). In high-risk areas, the prevalence of the disease is estimated to be between five and six percent, and in the surrounding areas, the prevalence is estimated to be around one percent (Miller 2000). Outside the U.S., CWD has been diagnosed on game ranches in Saskatchewan, Canada since 1996. In 2001, the Canadian Food Inspection Agency (CFIA) confirmed that a wild mule deer in Saskatchewan had tested positive for CWD (Venter 2001), representing the first case of CWD in the wild animal population in Canada (Canadian Food Inspection Agency Animal Products Animal Health and Production 2001).

CWD is characterized by a progressive loss of body condition and by neurologic changes. It is believed that the infection is passed between animals *via* horizontal transmission. Most of the animals dying naturally from the disease are between the ages of two and seven years. Polymorphism in the PrP gene in elk has been associated with changes in the susceptibility to infection (O'Rourke 1999). Extensive nationwide surveillance started in 1997 in an effort to better understand the geographic location and magnitude of the problem (U.S. Department of Agriculture 2001a).

A small number of deer are commercially slaughtered for human consumption every year (U.S. Department of Agriculture 1998). These animals are usually younger than 1.6 years and are not likely to be recycled into ruminant feed (Floyd 2001). Hunters often process the cervids they kill themselves or use local butchers. In these cases, high-risk materials may be further processed at a prohibited rendering facility. Game deer are fed with protein supplements that contain small amounts of vegetable supplemental protein (Floyd 2001).

Although CWD has been transmitted to cattle *via* i.c. inoculation, transmission by natural routes to cattle is very unlikely to occur (Hamir 2001). For example, cattle orally inoculated with CWD infected brains have not shown any evidence of infection (Williams 2001). *In vitro* experiments investigating the conversion of bovine PrP^{c} by PrP^{CWD} suggest evidence of a molecular barrier limiting the susceptibility of cattle (Raymond 2000). Moreover, even if CWD could be transmitted to cattle, it is not clear it could cause BSE. For example, preliminary analysis of intracerebral inoculation of cattle with CWD revealed no typical TSE lesions in brain but did reveal the presence of PrP^{sc} by immunuhistochemistry, Western Blot, and electron microscopy in three animals 22-27 months post-inoculation (Hamir 2001).

Even if CWD exposure can cause BSE, it is likely that the species barrier is substantial. *In vitro* assays have suggested that homology between an infectious and endogenous PrP molecule influences the rate and likelihood of conversion from PrP^{c} to PrP^{sc}, allowing the TSE disease to develop in the host (Priola 1994). *In vitro* studies have also shown differences between bovine PrP and PrP^{CWD} and these differences may represent a substantial species barrier. For example, Raymond et al. (Raymond 2000) reported that the cell-free conversion efficiency between bovine PrP^{c} and PrP^{CWD} is between five and twelve orders of magnitude weaker than inter-cervid transmission. This finding suggests that the species barrier may be between 10^{5} and 10^{12} (Raymond 2000).

Ascertaining the potential risk posed by oral exposure to CWD is further complicated by the following sources of uncertainty. First, there are no accurate statistics documenting the number or type of deer and elk killed by hunters. Second, the type of deer and elk that can be hunted in different geographic areas varies. Third, the disposition of deer and elk remains after slaughter is uncertain. Finally, the prevalence of the disease in all but the highest risk areas is unknown.

2.3.5 Chronic Wasting Disease: Lateral Transmission

Direct contact between cattle and cervids in regions where CWD is prevalent may provide another pathway by which cattle may become infected. Epidemiologic modeling suggests that among cervids, environmental contact provides a pathway for the spread of CWD (Miller 2000). Moreover, the prevalence of the disease under experimental conditions appears to be extraordinarily high. For instance, when deer within the endemic research facilities have been introduced into CWD negative deer herds, the disease quickly reaches a prevalence of 50% to 60% (U.S. Food and Drug Administration 2001a).

Nonetheless, there is no evidence that CWD can cause TSEs in cattle. As noted in Section 2.3.4, any such transmission would be limited by what appears to be a substantial species barrier. Moreover, cattle cohabiting with CWD infected deer and elk in a research facility in the endemic area have shown no evidence of infection (Williams 2001). Finally, targeted surveillance of cattle brains (by immunohistochemistry and histopathology) from endemic areas have failed to reveal the presence of CWD or any other TSEs (Gould 2000).

2.3.6 Mink

Transmissible Mink Encephalopathy (TME) is a rare disease known to occur only in farm-raised mink. Epidemiological studies have suggested that TME is a foodborne disease with an incubation period of between seven months and a year (Hartsough 1965). The disease is characterized by a long incubation period, a clinical course of several weeks, and neurological changes. Mink experience increased aggressiveness, hyperexitability, ataxia, and hyperaesthesia. Cases in Wisconsin and Minnesota were recognized on mink ranches as early as 1947. Outbreaks have been reported in Ohio, Canada, Finland, Germany, and Russia. Five outbreaks have been recorded in the U.S., affecting a total of 23 mink ranches (Hartsough 1965; Hadlow 1987; Marsh

1991). Three of the outbreaks in Wisconsin were associated with the use of fallen or sick cattle in mink feed (Hartsough 1965; Marsh 1991). However, there is no consensus over the source of disease initiating these outbreaks. The rancher involved in the Stetsonville, Wisconsin outbreak claimed that his mink were fed only dead stock and that they were never fed sheep.

In theory, transmission of TME to a bovine could cause BSE. Inoculation of cattle with TME *via* i.c. administration resulted in spongiform encephalopathy a short time later (Marsh 1991; Robinson 1995). In addition, cattle passage of TME remains pathogenic to mink when administered either orally or *via* i.c. (Marsh 1991). According to Marsh, these results suggest that the species barrier between mink and cattle may not be substantial. To protect against the possibility that TME might be transmitted to cattle, the FDA prohibits use of mink protein in ruminant feed. Mink are considered prohibited materials. The relatively small number of farmed mink, their small size, and recycling prohibitions make transmission of a prion disease from mink to cattle extremely unlikely.

2.3.7 Pigs

There is a theoretical risk that cattle could be exposed to a TSE as the result of consuming feed supplemented with porcine-derived protein. Moreover, the fact that federal regulations classify protein from pigs as non-prohibited increases the potential for cattle to be exposed to any infectivity they may harbor.

There are two potential sources of this exposure: a natural TSE that infects pigs (Section 2.3.7.1), and BSE-contaminated feed in the gut at the time the pig is slaughtered (Section 2.3.7.2). In practice, neither infectivity source will make a substantial contribution to cattle exposure because only a small portion of porcine-derived MBM is used as cattle feed. One reason for the limited use of porcine-derived protein in cattle feed derives in part from its price. For example, in May, 2001, the price of porcine-derived protein was $238/ton, compared to $177/ton for soy protein (Southern States Cooperative 2001). In addition, much rendered porcine protein is used in feed for pigs

The remainder of this section outlines additional factors that influence the importance of this potential source of TSE exposure among cattle. Because this source is unlikely to be significant, we do not address it quantitatively in our risk assessment (Section 3).

2.3.7.1 Potential Infectivity in Pigs due to TSE Infection

Pigs might become infected with a TSE as the result of any of the following possibilities:

- The existence of a porcine-specific TSE agent;
- A nonspecific TSE agent not yet adapted to pigs that has an incubation period that is longer than the life of the pig; or
- The spontaneous misfolding of the prion protein leading to a spontaneous TSE case in porcine.

Consumer groups in the U.S. have expressed concern that it may be possible for a pig to become infected with a TSE. This concern stems from a 1979 incident in which one of 60 pigs presented with clinical neurologic signs, neurological degeneration, and gliosis on histopatological examination (Hansen 1999). However, further testing showed that the lesions were not patognomonic of spongiform encephalopathy (Detwiler 2000). Moreover, the animal in question was young, a factor that is inconsistent with the TSE diagnosis. SSC (Section 4.1a in (European Union Scientific Steering Committee 1999b)) concluded that these factors argue against the presence of an unrecognized spongiform encephalopathy in pigs in the U.S.

Other evidence also suggests that the existence of a porcine-specific TSE agent is unlikely. No naturally occurring TSE has ever been reported in pigs (European Union Scientific Steering Committee 1999b). Moreover, pigs inoculated orally with BSE have not developed disease (European Union Scientific Steering Committee 1999b). Experimental inoculation of pigs with different strains of Kuru *via* parenteral administration did not lead to spongiform encephalopathy 52 to 76 months post inoculation (Gibbs 1979). A similar result was reported for pigs challenged with scrapie (European Union Scientific Steering Committee 1999b) up to 63 months after inoculation. Pigs have also been challenged with brain material from cattle naturally infected with BSE by combined i.c., i.p., and i.v. routes (Dawson 1990). This experiment is still ongoing but preliminary results show that seven of ten pigs developed spongiform encephalopathy (Ryder 2000). Experience in the UK during the BSE outbreak, when swine had significant exposure to BSE contaminated feed, suggests that the disease did not cross the species barrier to infect pigs (MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) 2001b).

In an ongoing experiment in which ten pigs were subject to oral challenge with large amounts of brain from cattle naturally infected with BSE, none of the animals have developed clinical disease or neuropathologic changes. The oral challenge consisted of homogenized brain from confirmed cases of BSE. Each pig received 1.2 kg of brain divided into three doses administered at intervals of one week. Mouse bioassays of neural and non-neural tissues from pigs killed at 84 months post inoculation were initiated in October and November, 1997 and were completed in May, 2000. As of the drafting of this report, there is no evidence of residual infectivity in any of the tissues. These findings may indicate that the species barrier between pigs and cattle is higher than the species barrier between cattle and humans or between cattle and other animals that have developed spongiform encephalopathy after exposure to BSE-contaminated MBM.

The fact that a naturally occurring spongiform encephalopathy has never been reported in pigs may indicate that pigs are particularly resistant to this type of disease. That is, the species barrier between pigs and other species may have prevented the transmission of natural disease to pigs. For example, it is very likely that pigs in the UK were exposed to substantial doses of contaminated MBM before the implementation of the UK feed ban. Although most pigs are slaughtered at a very young age, there are a significant number of sows and boars that usually live until age four. The fact that parenterally challenged animals developed disease 17 months post inoculation suggests that these four year old animals were sufficiently old to develop disease. Other species serve as examples. Marsh et al. (1969) noted the recovery of TME from the spleen of one chicken, and from spleen, caecom, tonsils, and bursa of Fabricius after i.v. inoculation. Race and Chesebro (1998a), have shown that after inoculation of mice that either did or did not express the prion protein (PrP) gene with the hamster scrapie strain 263K, no clinical disease was produced in mice. However, infectivity found in the brain and spleen of mice expressing the prion protein was capable of causing disease in hamsters but not in mice. Hill et al. (2000), have shown the possible presence of subclinical TSE in certain animals by demonstrating that a strain of hamster prions thought to be nonpathogenic for conventional mice leads to high levels of prion replication in such mice without causing clinical disease. Alternatively, it is possible that cases in pigs in the UK have gone unnoticed.

Finally, even if pigs could become infected with a TSE, most are slaughtered at a young age, making it unlikely that the disease would have time to generate more than a small amount of

infectivity. For example, in 1998, more than 95% of the pigs slaughtered in the U.S. were no older than six months (U.S. Department of Agriculture 1998).

2.3.7.2 Potential Infectivity in Materials Consumed by Pigs

Even if pigs do not become infected with a TSE, contaminated material may be present in their digestive tract when they die. In particular, if feed administered to pigs contains cattle-derived MBM that is contaminated with BSE, pigs could harbor BSE in their alimentary tract. The following discussion outlines several factors suggesting that the potential is limited for BSE to be recycled through the guts of pigs.

First, most pigs are not exposed to cattle-derived MBM because there are many other economical sources of protein. For example, in the U.S., soybean meal is usually the most economical source of high quality protein available for porcine diets. It is comparable to animal proteins in terms of the quality of its amino acid components and can be used as the only protein source in most swine diets. Other sources of proteins fed to pigs include porcine MBM, peanut meal, fish meal, cottonseed meal, canola meal, sunflower meal, and raw soy beans. The amount of protein added to feed varies based on the specific needs of the animal as it grows. MBM, blood meal, and plasma can comprise between 2.5 and 5 percent of feed for pigs between weaning and 60 days of age and during the animal's growing and finishing stages. Because it is not uniformly used, it is likely that approximately 80% of the pigs grown in the United States never receive MBM.

Second, even among pigs that do receive cattle-derived MBM, it is likely that little if any feed would remain in the GI system at the time of processing because pigs are usually sent to slaughter after restricting feed intake for 14 to 16 hours.

Third, due to the high water content of the GI tract, its contents are unlikely to be rendered.

Finally, if the gut contents are rendered, any BSE-contaminated material that does make its way back to cattle will have gone through rendering twice, thus providing an additional opportunity for infectivity to be destroyed by this treatment.

2.3.8 Poultry

Many of the same factors that make pigs an unlikely source of infectivity for cattle also make chickens an unlikely source. As a result, we do not quantitatively address this source in our risk assessment (Section 3).

As is the case with pigs, experimental data do not support the existence of a poultry-specific TSE. Experiments have subjected chickens to TSE challenge *via* both the parenteral and oral administration routes. The chickens challenged orally received five grams of brain from cattle confirmed to have BSE on three occasions at four, five, and six weeks of age. The chickens challenged i.c. received 50 µl of a 10% saline suspension of brainstem material from these cattle at one day after birth and one ml at two weeks after birth. No evidence of transmissible encephalopathy was found (European Union Scientific Steering Committee 1999b). In addition, there is no evidence to date of residual infectivity in any tissue (Detwiler 2000). Even if there were a poultry-specific TSE, the fact that chickens are typically slaughtered at an early age makes it unlikely that a prion disease would have time to develop.

Although chickens themselves appear to pose no substantial risk to cattle of exposure to a TSE, the use of chicken litter as a feed supplement could pose a risk (Public Citizen 2001) that should be investigated further. It is possible that cattle-derived protein feed supplements administered to chicken could contain BSE infectivity, and that BSE infectivity could pass through chicken and become available in cattle feed supplemented with chicken litter.

2.3.9 Recycled Products

Several products that can be used in ruminant rations have the potential of harboring infectivity. The FDA does not exclude from ruminant feed the following products derived from mammals:

- Plate waste: Inspected and processed meat products that have been cooked and offered for human consumption and further heat processed for feed (such as plate waste and used cellulosic food casings);
- Gelatin,
- Milk products (milk and milk proteins),
- Blood and blood products,

- Tallow, grease, fat, oil
- Aminoacids, dicalcium phospahate, and
- Mammalian protein, which consists entirely of porcine or equine protein.

2.3.9.1 Plate Waste

Plate waste is defined to be food products that have been inspected by the FSIS or an equivalent state agency, cooked, and presented for human consumption. Plate waste often comes from large institutions, such as amusement parks or hotels. It consists of food items that have been cooked and presented for human consumption. Plate waste can undergo any of the following heat treatments: conventional rendering, extrusion and cooking at 212 ^{o}F for 30 minutes (Swine Health Protection Act), pelleting at either 190 ^{o}F (internal temperature), or pelleting at temperatures similar to those of conventional rendering. This additional treatment using high temperature and pressure may further reduce the amount of infectivity that might be in this material.

There are approximately half a dozen processors of plate waste in the United States. The final product competes with grains in the formulation of feed, although at this time, it is not a cost-effective option because of the high processing costs.

Bakery products comprise approximately 90% of all plate waste going to animal feed. The remainder consists of eggs, dairy products, fish and other products of animal origin. Therefore, plate waste consists mostly of non-meat products (U.S. Food and Drug Administration 1997). Because plate waste is high in moisture content, vegetable proteins must be added (50% to 60%), and it must undergo further processing to aid the dehydration and extrusion process. The best estimate is that plate waste consists of between two and four percent bovine tissue. APHIS has expressed concern that plate waste could contain infective tissue, such as brain and spinal cord (U.S. Food and Drug Administration 1997), but such tissues are unlikely to be present in any substantial quantity in plate waste.

We assume that high risk tissues (*i.e.*, brain) are extremely unlikely to be included in plate waste. Plate waste might contain on occasion spinal cord contamination or spinal cord in a T-bone steak. Because T-bone steaks originate from younger cattle, the likelihood of carrying infectivity at the time of slaughter is extremely low. In addition, spinal cord is unlikely to be

included in the product (U.S. Food and Drug Administration 1997) because T-Bone steaks are unlikely to be included in plate waste.

In conclusion, current practices make the amount of infectivity in plate waste to which cattle could be exposed very small. Plate waste consists of little mammalian protein, and the tissues that are included in this waste are unlikely to contain BSE infectivity. Moreover, plate waste undergoes a substantial amount of heat treatment, which would further reduce the level of infectivity in this material.

2.3.9.2 Gelatin

FDA regulations allow the feeding of gelatin to ruminants because it has not been shown to harbor infectivity. SSC considers gelatin, amino-acids, and dicalcium phosphate to be safe if processing ensures that all material is subjected to degreasing, followed by acid and/or alkaline treatment, heating to 120°C, and then heating to 138-140°C for four seconds. (European Union Scientific Steering Committee 1998b). We assume that recycling this material poses little risk of exposing cattle to BSE.

2.3.9.3 Milk

FDA regulations do not prohibit the use of milk in cattle feed. No infectivity has been detected in milk or in the udder (mammary gland) of cows (as measured by the mouse bioassay) (Wells 1998). At this time, there is no evidence that any of the TSEs can be transmitted through milk.

SEAC considers milk to be safe and has concluded that there was no reason, following the interim results of a cohort study, to change this position (Spongiform Encephalopathy Advisory Committee 1999a; Spongiform Encephalopathy Advisory Committee 1999b). The World Health Organization has also issued a statement that concludes that milk is safe, most recently in June 2001 (WHO 2001). SEAC reviewed the processing and use of milk in the light of research implicating lymphocytes in the pathogenesis of TSEs (Spongiform Encephalopathy Advisory Committee 1999a; Spongiform Encephalopathy Advisory Committee 1999b). The Committee noted that there was no evidence of infectivity in the spleen or lymph nodes of cattle infected with BSE. In an opinion on the vertical transmission of BSE, the SSC also concluded there to be no reason to restrict the use of milk. However, SSC does recommend that as a

precautionary measure, milk from BSE-affected cows be kept out of the human food supply (European Commission 1999b). We assume that recycling this material poses little risk of exposing cattle to BSE.

2.3.9.4 Blood and blood products

No detectable infectivity has been found in blood or blood components of cattle infected with BSE (Wells 1998; Bradley 1999; Wells 1999; MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) 2001a). Unlike TSEs affecting other animals, infectivity has not been detected in spleen or lymph nodes of cattle experimentally infected with BSE during either the incubation period or after manifestation of clinical signs. Infectivity in blood has been found in sheep experimentally infected with BSE (Foster 1996). It is possible that in the cases of Scrapie, CWD, vCJD, and perhaps BSE in sheep, the lymphoid tissue plays an important role in the pathogenesis of the disease (Sigurdson 1999; Andreoletti 2000; Ironside 2000). Some have speculated that neuroinvasion can occur directly *via* peripheral nerves or *via* the lymphoreticular system and then subsequently the peripheral nerves (Glatzel 2001). Even if infectivity does exist in the blood of BSE-infected cattle, the total amount of infectivity is below the level of detection of the mouse bioassay. We assume that recycling this material poses little risk of exposing cattle to BSE. We do evaluate the potential for BSE infectivity to be present in blood as CNS micro-emboli (Section 3.1.2.3). In our sensitivity analysis, we characterize the effect of assuming inherent infectivity in blood at the level of detection (Section 3.2.2.1).

2.3.9.5 Tallow

The World Health Organization has concluded that because of the proteinaceous nature of TSE agents, they will tend to remain with the cellular residues of MBM during the extraction process, rather than being extracted with the lipids of tallow (WHO 2001). In addition, a rendering study funded jointly by the EU and MAFF in 1997 showed that tallow can be considered to be safe even if its treatment does not achieve the 133^{o}C/20 minutes/3 bars of pressure minimum treatment standard (Taylor 1997b). We assume that recycling this material poses little risk of exposing cattle to BSE.

2.4 Measures Taken to Protect Against BSE

Protecting against the introduction of BSE into a country requires policies that are based on prohibitions and restrictions. Regulations mainly prohibit the import of feed, feeding materials or animals from countries with BSE, and ban recycling of ruminant materials into ruminant feed. In countries with BSE, one common risk management strategy is the removal of infected tissues from the human food supply or animal feed. Minimizing the spread of disease is complicated because BSE animals may not show clinical signs of disease for an extended period after becoming infected and the agent cannot be deactivated readily once it contaminates feed. After describing these problems (Section 2.4.1), this section discusses actions taken in the UK to address its epidemic (Section 2.4.2), actions taken in Europe to prevent the spread of BSE beyond the UK (Section 2.4.3), measures taken in the United States to prevent the introduction of BSE (Section 2.4.4), and surveillance measures in the United States (Section 2.4.5).

2.4.1 General Issues Related to the Surveillance of BSE and the Deactivation of the BSE Agent

There is no pre-clinical or clinical test available to identify BSE in the field except when the disease is very near the end of its incubation period. Diagnosis in a live animal is based on clinical signs. Most commonly, the animal presents with changes in temperament, such as nervousness or aggression, abnormal posture, lack of coordination, difficulty in rising, or loss of body condition despite continued appetite. Specific clinical signs include abnormal head and ear position, apprehension and nervousness, apparent blindness, and exaggerated responses.

Microscopic examination is performed post-mortem and requires brain material preserved in formalin. This tissue is then stained and examined for the characteristic appearance of BSE specific changes. Microscopic examination is required by the EU Diagnostic Manual (European Commission 1999a; European Commission 2000) and by the Office International des Epizooties (OIE) (2000). The brain neuropathology of BSE resembles that of natural scrapie in sheep: astrogliosis, intracellular vacuolation affecting the gray matter neutropil and perikarya giving the distinctive appearance of spongiform changes, neuronal loss, and cerebral amyloidosis generally represent the most prominent changes (Wells 1991).

Other validated tests for postmortem diagnosis include:

- The detection of disease-specific brain changes by electron microscopy (scrapie associated fibrils, SAF);
- The detection of the abnormal form (PrP^{BSE}) of the host protein (PrP^{c}) by immunological means such as immunoblotting, immunohistochemistry (used as a confirmatory diagnoses by the EU); and
- Detection of infectivity in tissues by bioassay (usually i.c. injection into mice).

Newer tests have been developed that are capable of detecting pre-clinical cases and of screening animals before slaughter. These tests have high sensitivity and specificity for detecting and confirming BSE for diagnostic purposes and can detect disease in animals not showing typical signs at slaughter (European Commission 1999a). So far, the sensitivity and specificity of the newer tests have not been shown to be better than that of immunohistochemistry at similar preclinical stages of the disease (Detwiler 2000). The tests include:

- An immunobloting test based on the Western Blotting procedure for the detection of Pr^{Pres} using monoclonal antibody (Prionics);
- A chemiluminescent ELISA using polyclonal anti PRP antibody for detection (Enfer); and
- A sandwich immunoassay for PRP^{res} (CEA).

All of these tests are very rapid, with results obtained within 24 hours. Results from the Swiss surveillance system show that the Prionics test could be used to target populations other than those with clinical signs of the disease (Doherr 1999; European Commission 1999a; Schaller 1999). Although these tests can detect PrP^{res} before the animal shows clinical signs of the disease, it is not clear at what point during the incubation period the test would reliably yield positive results. It appears that their utility is primarily near the end of the disease progression.

There are no commercially available methods for detecting the BSE agent in food. On the other hand, food and food products can be screened to detect high risk tissues, such as brain in sausages (European Union Scientific Steering Committee 1999a; Lucker 2000), spinal cord in ground beef (Schmidt 1999; Schmidt 2001), or Advanced Meat Recovery (AMR) product (Kelley 2000; Schmidt 2001), and central nervous system tissue in the blood of animals stunned with pneumatic stunners (Garland 1996; Anil 1999; Schmidt 1999).

TSE agents are known for their capacity to survive severe environmental conditions such as desiccation, thermal extremes and UV exposure. Autoclaving, sodium hydroxide and sodium hypochlorite can achieve some level of inactivation but only under the most severe conditions. Currently there is no method to inactivate BSE that may be present in food for human consumption.

2.4.2 Actions to Address BSE in the UK

Soon after the BSE epidemic emerged in the UK, several measures were taken to decrease the incidence of the disease in animals and the potential exposure of humans to potentially infected meat products (MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) 2000). The disease became notifiable in 1988, requiring that all cattle suspected of having BSE be destroyed and sent for diagnosis. Upon confirmation of the role of animal feed in the epidemic in 1988, the UK banned the use of MBM as an ingredient in feed produced for ruminants. In September 1990, there was a ban on the use of Specified Bovine Offals (SBO) as an ingredient in feed stuff for any species. This legislation banned from the animal feed chain tissues identified as potentially harboring the highest concentration of infectivity. This determination was made based on knowledge of the distribution of infectivity in animals infected with scrapie (Hadlow 1980; Hadlow 1982; MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) 2001c). Feed-borne BSE infections continued even after the imposition of the feed ban, but at a much lower rate (Anderson 1996). As a result of these ever more stringent bans to reduce the recycling of infectivity, the annual incidence of BSE fell by 90% between 1992 and 1997. In March 1996, the government of the UK imposed a total ban on the use of mammalian proteins in feed produced for farm animals.

During the first few years following the initial identification of BSE in Britain, the epidemic was primarily an animal health concern. That focus reflected the experience with scrapie, which despite its presence in the human food supply for hundreds of years, had never lead to a known case of human illness. There was some concern given knowledge of the ability of scrapie to cross species under certain circumstances and as a result, precautionary measures were put into place to protect the human food supply, including a ban on the sale of brain, spinal cord, spleen, thymus, tonsils and intestines from cattle older than 6 months (materials designated as specified bovine offal or SBO) for human consumption.

In 1990 a domestic cat was diagnosed as suffering from a 'scrapie-like' spongiform encephalopathy. This event generated concern that BSE also might be transmissible to other species, including humans. Scientists theorized that if BSE were transmitted to humans, it would be likely to resemble CJD and suggested that surveillance be put into place to identify atypical cases or changing patterns of the disease. In 1990 The National CJD Surveillance Unit was set up at the University of Edinburgh in response to a recommendation from the Working Party on Bovine Spongiform Encephalopathy (also known as the SouthWood Committee) (UK CJD Surveillance Unit 2003). In 1994 the Spongiform Encephalopathy Advisory Committee (SEAC) judged the risk of transmissibility to humans to be remote only because precautionary measures had been put into place.

In 1996 a new variant of the human Creutzfeldt-Jakob disease (now known as variant CJD or vCJD) was announced in the United Kingdom. It was identified in a small group of people, all of whom were much younger than most individuals who develop CJD (Will 1996; CJD Surveillance Unit 2001). These cases shared clinical and neuropathological features that differed from those of CJD. As of August 31, 2001, the total number of definite and probable cases of vCJD reported in the United Kingdom was 106, including 12 probable vCJD deaths for which neuropathological confirmation will never be possible (CJD Surveillance Unit 2001). Outside the UK, four vCJD cases have been reported in France, one in Ireland, and one in Hong Kong. Clinical, pathological and molecular studies provide compelling evidence that the agent that causes vCJD is the same as that which causes BSE (Collinge 1996; Will 1996; Bruce 1997; Hill 1997; Scott 1999).

Following the announcement by the UK government that there appeared to be a link between BSE and vCJD, several additional measures were taken to reduce human exposure to the infective agent. SEAC recommended in March, 1996 that carcasses from cattle aged over 30 months be deboned and that all obvious lymphatic and nervous tissue be removed in licensed plants and that the trimmings be classified as SBO. For practical reasons, the ban was extended to apply to all material from animals more than 30 months of age. When the pathogenesis study suggested potential BSE infectivity in dorsal root ganglic, a ban on sale so of bone-in-beef was introduced. The ban on retail sales of bone-in beef was lifted in 1999 following a continued decline in the incidence of BSE and the implementation of other control measures to protect against the introduction of BSE into the human food supply.

Among other measures introduced were the compensation of producers for the loss of animals due to regulations related to the protection of public health and the eradication of BSE. Regulations aimed at eradicating BSE included the "selective cull" (implemented in 1997), which targeted for eradication animals that were the same age as an identified BSE case from their herd. Another regulation aimed specifically at protecting human health was the so-called "over 30 month scheme" (implemented in 1996), which prohibited the sale of beef from cattle over the age of thirty months for human consumption. The rational for this ban was that cattle over the age of 30 months could carry potentially substantial levels of infectivity in different tissues without having yet developed clinical signs of the disease. A complete chronology of the measures and regulations implemented in the UK following the initial outbreak of BSE in 1986 can be found at: http://www.defra.gov.uk/animalh/bse/.

2.4.3 Actions to Address BSE in Europe

Other countries that have reported cases of BSE imported either animals or animal feed from the UK. Schreuder *et al.* (1997) discussed the risk that such imports may have posed. It is estimated that a total of 58,000 adult breeding cattle were exported from the UK between 1985 and 1990. The UK also exported MBM during the years that the BSE agent is thought to have been present in rendering products from the UK. For example, because the price of MBM in England fell in 1989, exports of MBM doubled during that year (*e.g*, 15,000 tons of MBM were exported to France alone in 1989) (Taylor 1997a).

Cases of BSE have been reported in several countries. Some of these cases occurred in animals directly imported from the UK or in cattle fed with feed containing UK MBM. Following export of the disease from the UK, small epidemics were observed in Northern Ireland, Switzerland, France, and Portugal, presumably caused by the rendering and feeding of material from diseased animals. Few countries in Europe have not reported any BSE cases. Because the detection of BSE depends on the characteristics of the surveillance system, the absence of a reported case does not rule out the presence of the disease.

Due to the potential spread of BSE to other countries, the European Union and other international organizations have taken a wide range of measures to protect human and animal

health and to stop the spread of the epizootic (European Commission 2001). Some of the most important measures that have been taken by the international community include the following:

- The World Health Organization (WHO) in 1996 recommended that all countries ban the use of ruminant tissues in ruminant feed.
- In 1996, the European Commission banned the export of British live cattle, beef, semen, embryos, and all products produced from slaughter in the United Kingdom.
- The Office International des Epizzoties (OIE), the world organization for animal health, has codified the requirements for member countries to be considered BSE free. A risk assessment of the potential hazards is a pre-requisite.
- Feed bans in other countries: Since 1996, many countries have started to implement feed bans.
- In 2000, the Scientific Steering Committee of the EU reported the assessment of the geographical risk of BSE for 25 countries. This assessment considers the stability of the agricultural system and the likelihood of BSE occurring in the country (European Union Scientific Steering Committee 2000c).
- In 2000, the European Commission adopted the decision to remove specified BSE risk materials from the feed chain and from human food.
- In 2000, the European Commission prohibited the use of certain animal by-products, including MBM and blood meal (but excluding milk, or fish meal) from non-ruminants in feed for any farm animal species (effective January 1, 2001).
- In 2000, the European Commissions extended the SRM list to include bovine intestines.
- In 2000, the European Commission adopted the decision to test all cattle over 30 months of age. All animals over 30 months that cannot be tested or that test positive for BSE must be destroyed.

2.4.4 Measures to Prevent the Establishment of BSE in the United States Cattle Population

After more than 11 years of surveillance of high-risk animals, no confirmed cases of BSE have been reported in the United States. In cooperation with the USDA FSIS, the Animal and Plant Health Inspection Services (APHIS) has taken measures in prevention, education, surveillance, and response to reduce the risk of the introduction of BSE into the U.S. cattle population.

BSE became a reportable disease in the U.S. in 1986 (see Title 9 of the Code of Federal Regulations, Parts 71 and 161). As a result, USDA must be notified of suspect cases. Restrictions on imports from BSE countries have been in place since 1989, and active surveillance efforts began in 1990. As part of its surveillance effort, USDA has attempted to determine the disposition of cattle that were imported from the UK and the Republic of Ireland between 1980 and 1989. The Department continues to closely monitor the cattle in this group that have been located and are still alive.

In 1989, APHIS banned the import of all ruminants and restricted the import of certain cattle products from the United Kingdom and other countries where BSE was documented. In 1991, APHIS restricted the import of ruminant meat and edible products and banned most byproducts of ruminant origin from countries known to have BSE in a native animal (Title 9 Code of Federal Regulations, Parts 94.18 and 95.4). Countries whose products were restricted due to BSE prior to 1997 included: the UK (1989), the Republic of Ireland (1989), Switzerland (1990), Oman (1990), France (1991), and Portugal (1994). In 1997, the U.S. government prohibited the import of live ruminants and most ruminant products from all of Europe until a thorough assessment of the risks is conducted (U.S. Department of Agriculture 2003). In December, 2000, restrictions included a ban on the import of all processed animal proteins regardless the species, offal, tankage, ruminant fat, glands, processed fats and oils, and some tallow. The import restrictions in 2001 included a ban on the import of all ruminant meat, meat products and other edible ruminant products that were stored, processed or otherwise associated with a facility located in Europe or Oman. In 1990, APHIS developed a plan to respond to a BSE case in the U.S. This plan was updated by the APHIS-FSIS BSE working group in 1996.

FSIS performs *ante mortem* slaughter inspections at all federally inspected slaughter establishments and veterinary medical officers and food inspectors routinely evaluate animals for central nervous system disorders. Animals exhibiting suspicious CNS signs that may be indicative of BSE or any other reportable disease are withheld from slaughter. In such cases, Veterinary Services is notified. FSIS maintains a database containing information on *ante mortem* and *post mortem* condemnations triggered by these and other conditions.

The Food and Drug Administration (FDA) controls animal feed, thought to be the main exposure medium for the transmission of BSE among cattle. FDA prohibits the use of most

mammalian protein in the manufacture of animal feeds administered to ruminants (U.S. Food and Drug Administration 1997). This regulation is frequently referred to as the "feed ban." The regulation exempts the following products: blood and blood byproducts, milk products, pure porcine and pure equine proteins, plate waste, tallow, gelatin, and non-mammalian protein (poultry, marine, vegetable). Exempted products are considered to be non-prohibited material and all other mammalian protein is considered to be prohibited material.

The regulation specifies procedures for those renderers, protein blenders, feed manufacturers and distributors that produce prohibited material, non-prohibited material, or both. Firms must keep records on the origin of the materials and label prohibited material "*Do not feed to cattle or other ruminants*." Prohibited materials intended for export are exempt from this requirement and instead must be labeled "*For export only*." FDA requires that renderers, blenders, feed manufacturers and distributors selling both restricted and unrestricted products ensure that the two types of proteins are kept separate throughout processing. For example, there are minimum requirements for clean-out of the production line directly after the processing of prohibited material (*e.g.*, direct cleaning and flushing) before subsequently manufactured material can be considered to be non-prohibited.

In early January, 2001 FDA released results of a compliance survey of 9,947 rendering and feed mills. The data demonstrated that there is significant noncompliance with the feed ban in segments of the feed industry (U.S. Food and Drug Administration 2001b). Table 2-3 summarizes the results of this survey.

Table 2-3
FDA Update on Ruminant Feed (BSE) Enforcement Activities (January 10, 2001)

Practice	Proportion of Firms in Compliance		
	Renders[a]	FDA Licensed Feed Mills[b]	Feed Mills not licensed by FDA[c]
Firms whose products were labeled with the required caution statement	84%	80%	59%
Firms with a system to prevent commingling of prohibited and non-prohibited products	72	91	74
Firms that followed record keeping regulations	96-98	98	91

Notes

a. For Renderers --Total number of inspections – 239, firms handling prohibited material – 180.

b. For FDA Licensed Feed Mills – 1,240 total, inspected–- 846. Of those feed mills inspected, 347 were handling prohibited material.

c. For Non-FDA Licensed Feed Mills – 4,344 inspected (FDA does not know the total number because they are not required to be licensed by the Agency, but the number could be between 6,000 and 8,000). Of those feed mills inspected, 1,593 were handling prohibited material.

The FDA has also taken steps to reduce the risk of contaminating with BSE pharmaceuticals that contain or are produced with materials of bovine origin. These steps include the following:

- In 1992, FDA recommended the use of scrapie/BSE-free sources for materials used in dietary supplements.
- In 1993, FDA recommended that cell lines used for biologics be BSE-free.
- In 1993, FDA requested that bovine sourced materials, except gelatin, used in manufacture of regulated products be restricted to BSE-free countries.
- In 1994, FDA requested that bovine derived material for use in cosmetics and dietary supplements not be sourced from BSE countries.
- In 1996, FDA recommended the withdrawal of plasma and plasma products made from pools of persons who later developed CJD.
- In 1997, FDA requested more restrictions on the use of bovine gelatin originated from BSE countries.

- In 1999, FDA recommended deferring the use of blood from donors with more than six months cumulative residence in the UK during the period from 1980 to 1996.

2.4.5 BSE Surveillance in the United States

In addition to measures intended to mitigate the risk of BSE becoming established or propagated in the United States, the federal government has also implemented a surveillance program to help identify BSE as soon as possible if it were introduced into the U.S. Since 1986, when BSE became reportable in the U.S., USDA has encouraged the submission of brain tissue samples for testing, and has engaged in educating practitioners to look for and recognize the disease. Brain submissions are examined histologically, and immunohistochemistry has been used since 1993. USDA is considering the incorporation of rapid diagnostic tests for targeted surveillance in the future (Detwiler 2001). Between 1990 and July 2001, over 13,900 brains were examined. For each of the last six years, the rate of surveillance has been approximately double the OIE standard (OIE 2001). In 2000 and 2001, the number of brains examined was more than five times the OIE standard.

Animals targeted for surveillance by APHIS include cattle exhibiting signs of neurologic disease in the field (*i.e.*, prior to being brought to slaughter), cattle condemned at slaughter for neurologic reasons, rabies-negative cattle submitted to public health laboratories, neurologic cases submitted to veterinary diagnostic laboratories and teaching hospitals, and a sampling of cattle that are non-ambulatory ("downer cattle") at slaughter. Although the signs exhibited by non-ambulatory animals are generally not typical of BSE, some have suggested that these animals are a high-risk population based on anecdotal information that a TME outbreak could be linked to feeding practices involving non-ambulatory cattle (Marsh 1991). In addition, U.S. cattle inoculated i.c. with North American scrapie strains developed signs inconsistent with BSE signs manifest in affected animals in Europe but similar to those of non-ambulatory animals (Cutlip 1994; Cutlip 1997). The APHIS surveillance approach takes into account regional differences in the movement of animals. For example, based on movement patterns, the U.S. is divided into eight regions where adult cattle would typically live and exit the production system. Based on the adult cattle populations in each region, APHIS has calculated regional surveillance goals for BSE based on international standards as if each region were an individual country.

In addition to surveillance for the presence of BSE, The Centers for Disease Control and Prevention (CDC) monitors the incidence of CJD in the U.S. by analyzing death certificate information from multiple-cause-of-death data compiled by the National Center for Health Statistics. This information also is used to search for possible cases of vCJD in the U.S. As of September 2001, no such cases had been identified in the United States. Nor had any change in the incidence of CJD been observed.

3 Methodology

This section has four parts. Section 3.1 describes the simulation model developed as part of this analysis to quantify the impact of introducing BSE into the U.S. cattle population on both animal health and on potential human exposure to contaminated food products.

The description of the model in Section 3.1 reflects assumptions that are part of our "base case" scenario. This scenario represents the present state of the U.S. cattle population, along with government regulations and prevailing agricultural practices.

Section 3.2 describes the univariate sensitivity analysis and uncertainty analysis conducted to determine how changing various assumptions influences the model's predictions.

Section 3.3 describes how we used the model, with its base case assumptions, to evaluate the impact of alternative sources of infectivity on the U.S. given current conditions. These sources include spontaneous BSE, the import of from 1 to 500 BSE-infected cattle, the import of contaminated feed, domestic scrapie, chronic wasting disease, TSEs in domestic mink, pigs, and chickens, and recycled food waste.

Finally, Section 3.4 evaluates alternative scenarios, including Switzerland during the period when it is thought that country first imported BSE-infected animals. That evaluation, which compares empirically reported clinical BSE cases in Switzerland during the period 1985 to 2001 to the corresponding number of clinical cases predicted by the model, serves as an indicator of the model's plausibility. Section 3.4 also describes our methodology for evaluating the possibility of spontaneous BSE in the U.S. prior to the 1997 feed ban, the impact of importing infected cattle from the UK during the 1980s, and the implementation of various risk management strategies in the U.S.

Unless otherwise noted, all simulation scenarios were run 5,000 times. Appendix 4 discusses the numerical stability of the results.

3.1 Simulation Model and Base Case Assumptions

The simulation model consists of four components, as illustrated in Figure 3-1. The first component (Section 3.1.1) characterizes the lifecycle of cattle in the U.S., quantifies the potential

infection of animals at different points during this cycle, and characterizes their ultimate disposition (slaughter, death due to natural causes followed by either disposal or rendering, and death due to BSE infection followed by either disposal or rendering). The second component of the model (Section 3.1.2) describes how animals sent to slaughter are processed. Tissue may be disposed of, sent to rendering, or prepared for potential human consumption. The third component of the model (Section 3.1.3) characterizes the disposition of material sent to rendering. That material may be eliminated from further consideration (*e.g.*, because it will be disposed of, exported, or used to produce feed for animals other than cattle) or end up in feed that is administered to cattle. The final component of the model (Section 3.1.4) quantifies infectivity in food available for potential human consumption.

Detailed information on the parameters described in this section appears in Appendix 1. As described in that appendix, each parameter is defined as a table (although it may be a table containing only a single entry). These tables, in turn, are grouped into files. In our discussion below, we identify the name of the parameter table and the name of the file containing that table for each assumption discussed.

3.1.1 Cattle Population Dynamics

Figure 3-2 further details the cattle population dynamics component of the simulation model. In particular, this component describes the rate at which cattle are born, the rate at which animals are slaughtered, and the rate at which they die of other causes. Cattle can become infected when they are born as a result of maternal transmission. Alternatively, they can be born uninfected but become infected later as a result of exposure to BSE-contaminated feed. Infected animals may proceed to the clinical stage of the disease. Alternatively, they may be slaughtered, or die of other causes before that happens. Likewise, animals displaying clinical signs may also be slaughtered or die of other causes, including BSE. Section 3.3 details the different ways in which BSE infectivity could be introduced into the U.S. cattle population.

The model includes a detailed characterization of the cattle population dynamics because many of the rates influencing disease prevalence depend on animal age, type, and gender. Rates depending on at least some of these factors include the rate at which healthy animals become infected due to consumption of contaminated feed (this dependence stems from the influence of age, type, and gender on the amount of meat and bone meal (MBM) animals consume, and the

influence of age on susceptibility to infection given a specified exposure), the rate at which animals are slaughtered, and the rate at which animals die of causes other than slaughter.

The remainder of this section summarizes the following base case assumptions: 1) the number of animals in the U.S. cattle population by age, gender, and type (*i.e.*, dairy or beef, destined for production or breeding), their birth rate, slaughter rate, and rate of death from other causes; 2) cattle consumption of bypass protein and blood meal by age, type, and gender; 3) the dose-response relationship for cattle orally exposed to BSE and the influence of age on this relationship; 4) the rate at which infected cows transmit BSE to their offspring; and 5) the incubation period for BSE (*i.e.*, the time between infection and the appearance of clinical signs) and the time until death following the development of clinical signs.

3.1.1.1 Size of Cattle Population, Birth Rates, Slaughter Rates, and Rates of Death due to Other Causes

The simulation developed for this analysis requires specification of the number of animals by age in months for each gender within each of three animal types: dairy, beef, and beef reproductive animals. This last group represents those beef cattle that live beyond the age of 24 months for the purpose of providing beef calves. Base case values for the cattle population size appear in the <initsize> table of the genesisVisitor file (see Appendix 1).

Developing this information was complicated because available data sources do not break down the age distribution for cattle in sufficient detail, and in some cases, combine groups that must be characterized separately for the simulation. For example, statistics published by the FSIS Animal Disposition Reporting System (ADRS) (U.S. Department of Agriculture 1998) report the slaughter rate for dairy and beef cows combined, rather than specifying the slaughter rate for each group separately. The development of this information has been further complicated because some of the reported statistics do not appear to be consistent with each other. For example, as explained below, the reported number of steers and heifers slaughtered is consistent with birth rates that imply a total cattle population of approximately 140 million, rather than the true value of approximately 100 million.

When forced to diverge from reported statistics for the purpose of maintaining internal consistency, we do so in ways that minimize the impact of distortions on the validity of the simulation results. In the example described in the preceding paragraph, our inflation of the U.S.

cattle population should have a minimal impact on simulation results because the model-predicted rate at which BSE spreads does not in general depend on this statistic[3]. In particular, the assumed proportion of animals of each type (beef, dairy, or beef reproductive), gender, and age influences the probability of exposure to recycled protein. Because this relationship depends only on the relative size of the different cattle groups, scaling their size by a constant factor does not influence the simulation's results.

Population size

The specific population values for each type/gender/age category were computed using spreadsheet software and the birth, death, and slaughter rates described in this section. The documentation in Appendix 1 (file genesisVisitor, table <initSize>) describes these computations. However, although each simulation begins with the population distribution specified in Appendix 1, the model alters these values to reflect the simulated impact of birth, death, and slaughter. As a result of these influences, the initial population of approximately 140 million decreases to and stabilizes at approximately 130 million during execution of the simulation. As noted above, this change has a very limited impact on the simulation results.

Slaughter Rate

Animals are removed from the cattle population for slaughter at different rates depending on type, gender, and age. Dairy cows are culled as they age primarily when their reproductive and production capacity become inadequate. The base case assumptions for the slaughter rates are based on statistics recorded by USDA and are detailed in the <rateSlaughter> table, which is located in the rateSlaughter file (see Appendix 1).

Death Rate for Reasons Other Than Slaughter

The so-called "natural death rate" may be potentially important because some fraction of these animals, also referred to as animals that "die on the farm," are sent to rendering. Animals that die on the farm due to BSE infection have the maximum level of infectivity and therefore introduce the possibility that a substantial amount of BSE contamination could contaminate

[3] The possibility of spontaneous development of BSE is the one exception to this generalization because its rate is proportional to the size of the population. By overstating the size of the population, we have therefore overstated the

rendered products. The base case assumes that animals with BSE will live only 2 to 6 months after reaching the clinical stage of the disease. The natural death rates assumed by the base case are specified in the <probDeath> table, which is located in the deathVisitor file (see Appendix 1).

Birth rate assumptions

The base case assumes that female cattle can calve between the ages of 24 and 180 months. During that time, they produce a calf once every 12 months on average. Documentation accompanying the discussion of the birthVisitor file (see Appendix 1) explains the basis for these assumptions.

3.1.1.2 Proportion of Animals That Die on Farm that Are Rendered

The base case assumes that 85% of the animals that die on the farm (*i.e.*, before they are sent to slaughter) are rendered. This assumption is specified by parameter table <farmdeathdisp> in parameter file misc.

3.1.1.3 Cattle Consumption of Bypass protein and Blood Meal

MBM is one supplement for livestock feed, although in the U.S., other sources are also used, especially vegetable protein derived primarily from soybeans. The primary hypothesis for the spread of BSE in the UK is that infectious materials was recycled through the rendering and feed production processes resulting in the subsequent exposure of cattle. The amount of bypass protein-supplemented feed consumed by an animal, and hence its potential exposure to MBM, depends on the animal's type, gender, and age. Dairy cows receive the greatest amounts of supplemental bypass protein. Because the base case assumes that there may be breaches of the FDA feed ban (Section 3.1.3), some exposure can occur as the result of exposure to feed if BSE is present in the U.S. The <consumption> tables in files proteinInfector and bloodInfector respectively detail our assumptions for cattle consumption of bypass protein and blood meal.

3.1.1.4 BSE Dose-Response

The dose-response function for BSE quantifies the probability that an exposed animal will become infected with BSE as the result of ingesting contaminated materials. The dose is

---

potential impact of spontaneous disease, should it exist (see Section 3.4.2 in this report).

quantified in terms of the number of susceptibility-adjusted ID_{50}s ingested. The susceptibility-adjusted ID_{50} dose equals the product of an age-specific susceptibility factor and the number of unadjusted ID_{50}s ingested. The base case assumes that the dose response is linear up to an exposure level of 2.0 susceptibility-adjusted ID_{50}s, with an infection probability of zero at an exposure level of zero, and an infection probability of 1.0 at an exposure level of 2.0 adjusted ID_{50}s. For example, an animal that ingests 1.0 susceptibility-adjusted ID_{50}s has a 50% chance of becoming infected. Note that an animal that ingests more than 2.0 adjusted ID_{50}s has a 100% chance of becoming infected. Figure 3-3 illustrates the straight-line dose-response relationship assumed as part of the base case, along with a hypothetical alternative sigmoidal dose-response relationship. The dose-response relationship is specified by table <probInfection> in the proteinInfector file.

Our relationship between susceptibility and age (see Figure 3-4) is based on the assumption that susceptibility peaks at age four months and that it declines exponentially thereafter at a rate of 85% per year, leveling off at approximately 10% of its peak value (de Koeijer 1999). Table <susceptibility> in file proteinInfector (see Appendix 1) details this relationship. Section 2 provides further background on susceptibility.

3.1.1.5 Maternal Transmission

Although there is no direct evidence of BSE transmission from cow to calf, it is assumed to have occurred when a calf born to a cow incubating BSE contracts the disease in the absence of any other known sources of BSE exposure. Section 2.2.1 reviews evidence of maternal transmission for other TSEs, with the best evidence from scrapie (Kimberlin 1990; Foster 1992; Elsen 1999), and for BSE (Donnelly 1997a; Donnelly 1997b; Ferguson 1997a; Wilesmith 1997; Donnelly 1998). The base case assumes that calves born to infected cows during the last one-sixth of the incubation period will become infected with 10% probability. Text accompanying table <maternalContagiousPoint> in file sickBovine and table <probTrans> in file birthVisitor (see Appendix 1) further documents the basis for these two parameter values.

3.1.1.6 BSE Incubation Period, and Time Until Death Following Onset of Clinical Signs

The base case assumes that the duration between infection and manifestation of clinical signs follows a distribution inferred by Ferguson *et al*. (1997b) from data collected in the UK.

The density is right-skewed with a median of approximately four years. The 5th percentile is approximately 2.5 years, the median is approximately four years, and the 95th percentile is approximately seven years. Table <clinicalDate> in file sickBovine (see Appendix 1) further details this distribution.

The base case assumes that the time between the manifestation of clinical signs and death is uniformly distributed between 2 and 6 months (Heim 2001). Table <clinicalDuration> in file sickBovine (see Appendix 1) documents this assumption.

3.1.2 The Slaughter Process

If an animal with BSE is slaughtered, some practices can contaminate tissues destined for potential human consumption with BSE infectivity. In addition, many tissues not used for human consumption go to rendering and may become available to infect other bovines (Section 3.1.3). This section describes the base case assumptions for the slaughter process (Figure 3-5). It also describes ways in which infectivity can be diverted from uses that may result in either human or bovine exposure.

3.1.2.1 Level of Infectivity and Distribution of Infectivity Throughout the Carcass

The amount of infectivity that becomes available for human consumption or ends up being recycled into cattle feed depends in part on the total amount of infectivity in a slaughtered animal and how that infectivity is distributed through its carcass. Our model assumes these factors depend on the amount of time that has passed since the slaughtered animal became infected. Tables <organDistribution> and <totalInfectivity> in file materializer (see Appendix 1) details our base case assumptions.

Our description of the distribution of infectivity among the tissues of an infected animal is based on results from the pathogenesis experiment (Wells 1998; Wells 1999), as interpreted by SEAC (February, 1998). This experiment measured the infectivity in each of 44 tissues and fluids following experimental infection of cattle with BSE. The experiment found infectivity in the small intestine from months 6 to 18 months post infection, with no detectable infectivity in any other tissues. At the end stage of disease, (≥ 32 months post infection), infectivity was found in the brain, spinal cord, dorsal root ganglia (DRG), trigeminal ganglia (TGG), and again in the small intestine. We assume that findings of infectivity in bone marrow at one time point were

spurious, although we do investigate the potential for the disease to directly infect blood (so-called "inherent infectivity") (Section 3.2.2.1). Table 3-1 details our specific assumptions.

Note that these assumptions are based on an assumed incubation period of 36 months (as observed in the pathogenesis study). For animals with incubation periods of durations other than 36 months, the time periods post inoculation are scaled accordingly. For example, for an animal with an incubation period of 72 months, there is no infectivity in the brain prior to month 64 (*i.e.*, $32 \times 72 / 36$).

Table 3-1
Relative Infectivity of Specific Tissues Specified From an Infected Bovine
(Based on (European Union Scientific Steering Committee 1999a))[a]

Tissue	Fraction of Total Infectivity
Brain	No infectivity in cattle < 32 months post-inoculation (PI) 32 months PI and over: 64.1%
Trigeminal Ganglia	No infectivity in cattle < 32 months post-inoculation. 32 months PI and over: 2.6%
Other Head (eyes)	No infectivity in cattle < 32 months post-inoculation. 32 months PI and over: 0.04%
Distal Ileum	6-18 months post inoculation: 100% 18-31: No Infectivity 32 months PI and over 3.3%
Spinal Cord	No infectivity in cattle < 32 months post-inoculation. 32 months PI and over: 25.6% infectivity
Dorsal Root Ganglia	No infectivity in cattle < 32 months post-inoculation. 32 months PI and over: 3.8 % infectivity

Notes:

a. The post-inoculation time values in this table reflect the assumption that the incubation period is 36 months. See text for explanation.

The base case assumes that the total quantity of infectivity in an animal with BSE reaches its maximum level when the animal develops clinically detectable signs (*i.e.*, becomes "clinical") (see Section 3.1.1.6 for a discussion of the incubation period duration). Prior to that time, the total level of infectivity follows the pattern illustrated in Figure 3-6. The example in Figure 3-6 corresponds to the case in which the animal develops clinical signs 36 months after infection.

During the first five months of infection, total infectivity in the animal is around 0.1% of its maximum value, followed by an increase to around 2.5% of its maximum value between months 6 and 18 post infection. Up until this point, all infectivity is assumed to be in the gut. Starting in month 19, infectivity is assumed to be distributed among several tissues, with the bulk in the brain and the spinal cord, and the remainder divided among the gut, DRG, eyes, and TGG. At this time, total infectivity drops to zero but and then grows exponentially until it reaches its maximum level in month 36. For incubation periods other than 36 months, the model scales the horizontal (time) axis in Figure 3-6 proportionally. The total amount of infectivity in an animal with clinical BSE is assumed to 10,000 cattle oral ID_{50}s (European Union Scientific Steering Committee 1999a; Spongiform Encephalopathy Advisory Committee 2000; Gale 2001). Note that this value has not been adjusted to reflect age-specific susceptibility (see Section 3.1.1.4 and Appendix 1, Section 2.3.4).

3.1.2.2 *Antemortem* Inspection

Once the animal is at the slaughter facility, it is inspected for signs of disease. FSIS regulations require that for certain diseases, the whole animal is condemned at *antemortem* (AM) inspection (U.S. Department of Agriculture 1997). Condemned animals can be rendered or incinerated.

Animals not showing clinical signs at AM inspection are not likely to be condemned for BSE but could be condemned if they show signs of other diseases. The condemnation rates for animals not showing clinical BSE signs depend on age and gender. The rates used in the base case are based on data collected by FSIS for the year 1998 (see Table 3-2). In particular, the base case assumes that the AM condemnation rate is approximately 1% for animals less than one year of age, 0.01% for animals between the ages of one year and 31 months, and 0.2% for animals older than 31 months of age.

Table 3-2
Cattle Slaughtered and Condemned (1998)[a]

Cattle age group	Total Animals slaughtered	Total Animals Condemned	Probability not pass AM	Animals Condemned Postmortem	Probability that will not pass Postmortem
< 12 months	1,483,430	14859	0.0098	13,799	0.0092
12 to 24 months	32,690,003	2,349	0.0001	22,697	0.0007
≥ 24 months	7,815,074	21,906	0.0028	11,0172	0.0139

Notes:

a. Data from USDA's Animal Disposition Reporting System (U.S. Department of Agriculture 1998).

Animals that are condemned following AM inspection are usually rendered, although a small proportion are incinerated. The base case assumes that 98% of condemned animals are rendered and that the rest are incinerated. The likelihood that an animal condemned at AM inspection is rendered or incinerated is assumed to be independent of its BSE status. The means of disposal is important because animals that are incinerated cannot contaminate human food or animal feed.

Animals that do manifest the clinical signs of BSE can be identified by AM inspectors. It also is possible that farmers might hold back from slaughter animals with BSE signs to prevent the case from being discovered. It is difficult to estimate how effectively U.S. inspectors would detect an animal with BSE signs because the disease has not been detected in this country. The USDA has conducted training for inspectors to make them aware of these signs. The effectiveness of inspectors at detecting other CNS diseases could conceivably be used to estimate how effective they would be at detecting animals with clinical signs of BSE. In practice, however, the prevalence rate for these other diseases are often unknown. Our base case assumes that clinical BSE cases would be detected at AM inspection 90% of the time (see table <probPassAM> in file AMInspector). Because this value is highly uncertain, our uncertainty analysis evaluates the impact of using a wide range of values on the results of our simulation (see Section 3.2.2).

3.1.2.3 Stunning

Stunning humanely renders animals unconscious for slaughter. It is usually performed by mechanical devices, most commonly captive bolts that may or may not penetrate the skull. One

type of penetrating captive bolt is referred to as an “air-injected pneumatic stunner” because it injects a jet of air into the brain at the end of the cylinder stroke. Stunners that use air injection can deposit CNS tissue emboli in blood, heart, lung, and liver. Malfunctions in these devices both increase the probability that emboli will be created and the amount of emboli that will be deposited. However, based on our conversations with USDA personnel (in headquarters and in the field), individuals in the beef packing industry, and others, the base case assumes that air-injected stunning is not currently used in the U.S. cattle industry (see parameter table <probType> in file stunner). Other scenarios evaluating past practices do assume the use of air-injected pneumatic stunning (see Section 3.2.2).

There also is some concern that other stunning methods may produce CNS micro-emboli that could contaminate blood (European Union Scientific Steering Committee 2000e). The base case assumes that stunners not using air injection can create very small emboli that are found only in blood (see parameter table <emboli> in file stunner). The amount of emboli in the blood is not affected by whether the stunner malfunctions.

3.1.2.4 Exsanguination

Following stunning, animals are bled. Bovine blood can be processed for human consumption, processed to make blood meal that can be used in ruminant feed, rendered, or disposed of. The base case assumes that 15% of blood is made into blood meal that has the potential to be used in cattle feed (parameter table <probDestination in file feedTransporter). The base case also assumes that blood collected for human consumption is not contaminated with emboli.

Blood collected to produce meal for animal consumption may become contaminated with CNS tissue if some of that tissue drips from the hole created by the stunner. The base case assumes that air-injected pneumatic stunners generate this type of contamination with 30% probability (see parameter table <probDrip> in file stunner), and that when this contamination does occur, 4% of the infectivity in the brain ends up in the blood being collected (parameter table <fracDrip> in file stunner). The base case assumes that stunners that do not use air injection never cause this type of contamination.

3.1.2.5 Disposition of Brain

Following exsanguination, the head is removed from the carcass. USDA has mandated inspection of some parts of the head that are collected for human consumption (*e.g*, tongue). Because brain is the tissue with the greatest amount of infectivity in an animal with advanced BSE, the disposition of the head is important. There are no available data on the fraction of brains collected for sale as human food. The base case assumes that 1% of the brains are removed for potential human consumption in the U.S. and that the rest are rendered (see parameter table <probPassFood> in file foodInspector). Section 3.2.2.5 lists base case and worst case assumptions for other tissues.

3.1.2.6 Splitting and Aerosolization

After removal of the head, the carcass is split longitudinally with a saw to facilitate handling and further processing. When the carcass is split some spinal cord is aerosolized and can contaminate edible meat. Based on data from experiments that measured the amount of spinal cord associated protein deposited on the carcass during splitting (Harbour 2001), the base case assumes that approximately 0.001% (2.5 mg) of the spinal cord contaminates edible meat. The base case further assumes that additional carcass treatments, like washing and steaming, do not reduce the amount of contamination. Text accompanying table <fracAerosol> in file splitter (see Appendix 1) documents this assumption.

3.1.2.7 Disposition of the Spinal Cord and Dorsal Root Ganglia

The vertebrae of the animal are arranged in a column that houses and protects the spinal canal. Because spinal cord and the dorsal root ganglia (DRG), which are nerve ends emerging from the spinal cord, can contain BSE infectivity, their disposition influences the extent to which meat recovered for human consumption may become contaminated. The magnitude of this contamination and which selections of meat become contaminated depend on whether a mis-split occurs, whether the slaughter plant uses advanced meat recovery (AMR), and whether it removes the spinal cord from the carcass. The extent to which AMR product becomes contaminated is particularly sensitive to mis-splits because they can leave behind pieces of spinal cord encapsulated in the vertebral column that are processed by AMR. This section first discusses the frequency of mis-splits, the proportion of carcasses processed using AMR, and the proportion

from which the spinal cord is removed. Finally, it discusses how mis-splitting, AMR, and spinal cord removal influences contamination.

Mis-split frequency

Mis-splitting refers to the incomplete cutting of the spinal column with a saw. A mis-split occurs when the cut veers off the vertical and terminates at a point short of the cervical vertebrae (carcasses are split caudal to cranial). The likelihood that mis-splitting will occur depends on the size and age of the animal (*e.g.*, calves are more likely to be mis-split than bulls or cows) and the proficiency of the saw operator. The rate and extent of mis-splitting influences the potential for spinal cord from an infected animal to contaminate human food, primarily in the Advanced Meat Recovery process (Section 3.1.2.8). The base case assumes that among animals below the age of 24 months, mis-splits occur 5% of the time, whereas for older animals, mis-splits occur 8% of the time. Table <probMS_AMR_SCRemove> in file splitter (see Appendix 1) details estimates for the rate and extent of mis-splits.

The proportion of cattle processed using AMR

Once the carcass is split, the disposition of the spinal cord depends on whether or not the slaughter facility processes the vertebrae using advanced meat recovery (AMR). AMR machines process bones to recover meat remaining after the hand deboning process is completed. USDA rules allow the AMR product to be labeled as “meat”. Approximately 70% of fed cattle and 60% of cows are processed in facilities that use AMR (Sparks Companies 1999). The base case assumes that AMR is used to process no animals below the age of 12 months, 65% of animals between the ages of 12 and 23 months, and 60% of animals 24 months of age or older. Table <probMS_AMR_SCRemove> in file splitter (see Appendix 1) details estimates for the proportion of material processed using AMR.

Spinal cord removal – Plants that use AMR

An FSIS directive requires that the spinal cord be removed from the vertebral column before the backbones enter the AMR process. The base case assumes that spinal cords are removed with 98% probability in plants using AMR. Spinal cords removed in this manner are rendered. In the event that spinal cord is not removed prior to AMR, it can contaminate the AMR

product, although the probability of this occurring is small. In addition, if the carcass is mis-split, the spinal cord that remains encapsulated in the spinal canal (usually a small portion of the spinal cord) contaminates AMR product unless it is removed by facility personnel. Whether an AMR processing system is used depends on the size and age of the animal (*e.g.*, calves are not likely to go through AMR). The amount of spinal cord left behind that can contaminate edible meat also depends on the age and type of the animal (*e.g.*, for steers and heifers, the lumbar area does not go through AMR because T-bone steaks are more profitable). Table <probMS_AMR_SCRemove> in file splitter (see Appendix 1) details estimates for the proportion of spinal cords that are removed.

Spinal cord Removal – Plants that do not use AMR

If a facility does not use AMR, FSIS does not require removal of the spinal cord from the carcass. However, some slaughterhouses choose to remove it and send it to rendering. The base case assumes that spinal cords are removed with 50% probability in plants that do not use AMR. If the spinal cord is not removed, it remains in certain cuts of beef and is hence available for potential human consumption (*e.g.*, T-bone steak). In addition, spinal cord left in the carcass can contaminate the boning table. Finally, a small fraction of the spinal cords removed from steers and heifers are destined for human consumption. Table <probMS_AMR_SCRemove> in file splitter (see Appendix 1) details estimates for the proportion of spinal cords that are removed.

Fraction of spinal cord and DRG that contaminate meat recovered for human consumption

The DRG are firmly attached to the bones of the spinal column and are not removed even if the spinal cord is removed. The disposition of the DRG depends on the cuts of beef recovered for human consumption (which depend on the age of the animal) and on the use of AMR processing systems. For example, some cuts of meat from young animals, such as steers and heifers, might be sold with the vertebrae attached (*e.g.*, T-bone steaks), and in those particular circumstances DRG can reach the consumer. However, it is important to note that even if DRG reaches the consumer, it is unlikely to be consumed unless the bone is aggressively cleaned. In other regions of the vertebral column, DRG will remain attached to the bone because they are unlikely to be removed by standard deboning operations. The vertebrae and DRG from young animals are likely to be rendered. For older animals (*e.g.*, bulls and cows) that are deboned by hand, DRG will not reach the consumer and will instead be rendered.

If the spinal column is processed using AMR, the DRG are likely to contaminate the AMR product. For young animals, only a fraction of the vertebral column and DRG will be processed using AMR because parts of the backbone are contained in high value bone-in cuts of meat. For older animals, such as bulls or cows, all vertebrae are likely to be processed using AMR. If the facility does not process the spinal column using AMR, other technology, such as vibration or hand held knives (*e.g.*, Whizzard knives), are used to recover the remaining meat attached to the bones. Because of the location of the DRG and the presentation of the backbones on the boning table, these knives are unlikely to contaminate meat or ground beef with DRG or spinal cord.

Tables <fracDRGInMuscle>, <fracDRGInAMRMeat>, and <fracDRGInBone> in file splitter (see Appendix 1) detail the fraction of the infectivity in DRG that ends up contaminating muscle, AMR product, or remains connected to the bone, respectively. These values depend on whether a mis-split occurs, the use of AMR, and on whether the spinal cord is removed. Tables <fracSCInMuscle>, <fracSCInAMRMeat>, and <fracSCInBone> in file splitter (see Appendix 1) provide the corresponding assumptions for spinal cord contamination.

3.1.2.8 Postmortem Inspection

Organs and tissues from cattle passing AM inspection are inspected *postmortem* (PM) to ensure fitness for human consumption. FSIS regulations require that the whole animal be condemned when certain diseases are suspected, while for other diseases and conditions, only some tissues are excluded from use in human food. There are no visible characteristics of BSE cattle that can be detected at PM inspection. Nevertheless, the base case assumes that some infected animals or tissues from animals with BSE are condemned at PM inspection for reasons other than the presence of BSE. These condemnation rates have been measured and reported by FSIS (Table 3-2). The FSIS data specify rates by age and gender. The base case rates appear in Table <probPassPM> in file PMInspector (see Appendix 1).

3.1.2.9 Recovery of Material for Potential Human Consumption

After the carcass is split, meat for potential human consumption is recovered. Some potentially infectious tissues may be purposely recovered for potential human consumption. The base case assumptions for the proportion of various tissues recovered for this purpose are detailed

in Table <probPassFood> in file foodInspector. Not all tissue that is potentially available for potential human exposure is actually consumed. Rates of waste during distribution and in the home, portion sizes, and other factors will influence actual human exposure. Section 3.1.4 describes the basis for these assumptions.

3.1.3 Rendering and Feed Production

Rendering is a process that recovers useful materials like fat, tallow, and protein, by cooking the animal remains, separating the products, and by further processing and purifying the resulting meat and bone meal (MBM). MBM is a rendering product rich in protein that can be used as a feed supplement, among other uses. If the remains of an infected animal, including either a sheep with scrapie or a bovine with BSE, are made into MBM that is then fed to cattle, additional animals could become infected. Current regulations in the U.S. (U.S. Food and Drug Administration 1997) prohibit the feeding of mammalian derived protein to other ruminants with some exemptions. The feed ban does not restrict the use in ruminant feed of porcine protein, equine protein, ruminant blood, ruminant milk, plate waste, or gelatin. Other sources of protein, primarily of vegetable origin (*e.g.*, soy), are also widely used to supplement livestock rations. The extent of compliance with the feed ban in rendering and feed formulation influences the extent of possible cattle exposure to infectivity from a rendered diseased animal. Infectivity can also be eliminated as a result of using ruminant derived materials in ways that do not lead to any potential exposure among U.S. cattle (*e.g.*, export). Figure 3-7 illustrates our characterization of how materials flow through rendering plants, feed formulation plants, and to the farm.

3.1.3.1 Rendering Inactivation

Rendering may reduce the amount of BSE infectivity in material by subjecting it to heat and pressure. Different rendering systems (*e.g.*, continuous, batch, and vacuum) inactivate BSE or scrapie infectivity to different degrees (Taylor 1995; Taylor 1997b; Schreuder 1998). Table 3-3 quantifies the base case assumptions for the reduction in infectivity achieved by each technology and the proportion of animals rendered using each technology. The sensitivity analysis varies these proportions and the degree of inactivation achieved. The text in Appendix 1 accompanying table <renderFactor> in file renderer further documents these assumptions.

Table 3-3
Infectivity inactivation achieved and proportion of cattle processed by different types of rendering systems

Technology	Infectivity Inactivation Achieved (log base 10)	Proportion of cattle rendered
Batch	3.1 logs	5%
Continuous/fat added	2 logs	45%
Continuous/ no fat added	1 log	45%
Vacuum	0 logs	5%

3.1.3.2 Meat and Bone Meal Production

U.S. regulations recognize three types of rendering facilities, designated here as nonprohibited, prohibited, and mixed. A nonprohibited plant processes only porcine, equine or poultry (nonruminant species) and produces animal-based protein products that can be used legally in cattle feed. A prohibited rendering plant may process ruminant or mink raw materials, along with other sources of animal protein, and produces prohibited MBM that may not be used in cattle feed. Mixed plants produce both nonprohibited and prohibited MBM. These plants must use separate production lines or a common line with specified cleanout procedures. The base case assumes that 94.9999% of cattle remains are sent to prohibited rendering plants, 5% are sent to mixed plants, and 0.0001% are sent (incorrectly) to non-prohibited plants (see Table <probType> in file renderer)..

The base case assumes that cattle infectivity can reach bovines in several ways. Material from a prohibited rendering plant could be mislabeled and used in the formulation of cattle feed. Mislabeling could also occur in a mixed plant. A mixed plant could contaminate non-prohibited MBM by using incorrect source material or by failing to completely flush and clean shared processing machinery. A nonprohibited plant might contaminate their MBM by using prohibited source material although this is unlikely. Even if non-prohibited MBM is contaminated, the potential for bovine exposure is reduced because much of this MBM goes to uses other than cattle feed.

Table 3-4 describes the disposition of MBM infectivity based on the flow shown on Figure 3-7. Tables <probContaminate> and <fracContaminate> in file renderer respectively detail the probability that contamination will occur and the proportion of prohibited material that ends up in non-prohibited material. Table <probMislabel> details the probability that prohibited material will be mislabeled as non-prohibited. Finally, table <probDestination> in the

MBMTransporter file details the flow of material generated by renderers to various types of feed producers.

Table 3-4
The Flow of Infectivity Through the Rendering Process

Figure 3-7 Flow Reference Number	Label[a]	Description
1	To P MBM	Infectivity sent to rendering for prohibited MBM
2	SRM Elimination	Infectivity eliminated from potential use in animal feed or human food if specified risk material ban in place
3	Render Elimination	Infectivity removed through inactivation by rendering
4	Contam. NP MBM	Infectivity from P MBM that contaminates NP MBM in mixed rendering facilities
5	Mislabel P MBM	Infectivity in P MBM mislabeled as NP MBM
6	Out after Render	Infectivity in rendered material not used for livestock feed

Notes:

a. Entries in the "Label" column refer to the descriptors used in the output tables in the results Section (see Appendix 3A).

3.1.3.3 Feed Production

FDA feed ban regulations restrict use of prohibited MBM to feed manufacturers that produce only prohibited feed, or to manufactures that produce both prohibited and non-prohibited feed (mixed producers), so long as they adhere to procedures that minimize the risk of contamination. The base case assumes that at mixed facilities, prohibited feed could contaminate non-prohibited feed. In addition, the base case assumes that prohibited feed can be mislabeled in facilities producing both prohibited and non-prohibited feed. The documentation in Appendix 1 (see parameter file feedProducer) details these assumptions in further detail.

When recycled animal tissue (*i.e.*, blood meal or MBM) is used as a supplement to animal feed, the material can be divided among feed portions consumed by many cattle. The base case assumes that infectivity in the blood meal from a single animal is divided among 89 cattle

(see the documentation in Appendix 1 for the <numCowsReceiving> parameter table in the proteinInfector parameter file).

How widely infectivity in recycled protein is distributed is more complicated. If the infectivity does not contaminate non-prohibited MBM or non-prohibited feed, then it remains contained in a single "packet" (*i.e.*, a collection of material that travels together during processing) that can be divided among 89 cattle. However, if contamination occurs during the rendering process, then a portion of the infectivity is transferred to the affected non-prohibited MBM packet. That non-prohibited packet has the potential to exposure an additional 89 cattle. Finally, if contamination occurs during feed production, it is assumed that a portion of the infectivity is transferred to the affected non-prohibited feed packet. That non-prohibited feed packet likewise has the potential to expose an additional 89 cattle. Table 3-5 describes the flow of cattle infectivity through the feed production process. The parameter tables in files feedProducer and feedTransporter detail these assumptions.

Table 3-5
The Flow of Cattle Infectivity through the Feed Production and Use Processes

Figure 3-7 Flow Reference Number	Label[a]	Description
7	To P Feed	Infectivity in P MBM that goes to production of P livestock feed
8	To NP Feed	Infectivity in NP MBM reaching NP Feed
9	Contam NP Feed	Infectivity from P Feed that contaminate NP Feed in mixed feed mills
10	Mislabel NP Feed	Infectivity in P Feed mislabeled as NP Feed
11	To Blood	Infectivity reaching cattle feed through use of blood meal
12	Out After Feed Prod	Infectivity in livestock feed not used for cattle

Notes:

a. Entries in the "Label" column refer to the descriptors used in the output tables in the results Section (see Appendix 3A).

3.1.3.4 On Farm Feeding

The practice of administrating correctly labeled (*i.e.*, with the label "DO NOT FEED TO RUMINANTS") prohibited feed to cattle on the farm is referred to as "mis-feeding." The base case assumes that correctly labeled prohibited feed will be administered to cattle with a probability of 1.6%. Documentation accompanying the <probFeedOK> parameter table in the feeder file (see Appendix 1) explains our derivation of this estimate. Table 3-6. describes the flow of cattle infectivity on the farm.

Table 3-6
The Flow of Cattle Infectivity on the Farm

Figure 3-7 Flow Reference Number	Label[a]	Description
13	Misfed	Infectivity in materials fed to cattle in non-bovine livestock feed
14	To Cattle	Total infectivity reaching cattle through feed

Notes:

a. Entries in the "Label" column refer to the descriptors used in the output tables in the results Section (see Appendix 3A).

3.1.4 Potential Human Exposure

The base case assumes that humans can be exposed to BSE infectivity either by directly consuming infected cattle organs, such as brain, spinal cord, eyes, and distal ileum, or by consuming contaminated products, including meat or processed meat containing spinal cord or DRG, or organs containing CNS emboli, such as liver, heart, or kidneys. Table 3-7 details the assumed availability of these tissues (see parameter table <probPassFood> in parameter file foodInspector).

Table 3-7
Potential Human Exposure

Organs/tissues	Description/Assumptions
Brain	Brain is considered to be a variety meat and reaches the consumers labeled as such. The base case assumes that 1% of all cattle brains are potentially available for direct human consumption.
Spinal cord	Beef spinal cord is considered to be a variety meat and reaches consumers labeled as such. The base case assumes that 1% of the spinal cord is potentially available for human consumption.
Blood	The base case assumes that 5% of the cattle blood is potentially available for human consumption in meat food products (*e.g.*, sausages, blood pudding) and that the infectivity in blood is limited to the potential contribution from brain and spinal cord emboli. In our sensitivity analysis, we investigate the possibility that BSE disease itself also contributes to the infectivity in blood at a concentration that can be as high as the level of detection.
Distal Ileum	Distal ileum is considered to be a variety meat and reaches the consumers labeled as beef intestines. The base case assumes that 1% of the distal ileums are potentially available for human consumption. In the U.S., distal ileum does not reach consumers as natural sausage casings.
Contaminated Organ Meat	Brain and spinal cord are responsible for infectivity in organ meat when air-injected pneumatic stunning is used (not reflected in the base case). Liver, heart, and kidney are sold as variety meat. The base case assumes that 60% of the liver, 50% of the heart, and 25% of the kidneys are potentially available for human consumption.
Eyes	Bovine eyes are considered a variety meat and reach consumers labeled as beef eyes. The base case assumes that 1% of eyes are recovered and potentially available for human consumption.
Contaminated muscle meat	The base case assumes that spinal cord can contaminate edible meat during the splitting process. It is further assumed that other processes, such steaming or washing do not reduce this contamination. If this source of contamination were important, this assumption could be investigated further.
AMR	Total infectivity in AMR product is the sum of the contributions from spinal cord and DRG contamination. The amount of spinal cord contaminating AMR product depends on whether the spinal cord is removed, as required by FSIS regulation for plants using AMR, and on whether the carcass is mis-split.

Table 3-7
Potential Human Exposure

Organs/tissues	Description/Assumptions
Beef on bone	Total infectivity in beef on bone is the sum of the contributions from spinal cord contained in these cuts of meat and DRG attached to these bones. Spinal cord has the potential to reach consumers if it is not removed from the spinal column and if it remains attached to the backbone as bone-in steak. The base case assumes that about 30% of the backbones from steers and heifers are sold bone-in and that because these cuts do not undergo AMR processing, they retain the spinal cord. Although regulations do not require removal of the spinal cord from the backbones that do not undergo AMR (FSIS Directive 7160.2 1997), many slaughterhouses remove it anyway (Brewer 2000). Note that even if the spinal cord or DRG on beef on bone reaches consumers, this material is not likely to be eaten.
Trigeminal Ganglia	The base case assumes that the trigeminal ganglia (TG) does not contaminate cheek meat because it is located at the base of the cranium. Sensitivity analysis investigates the impact of assuming that TG contaminates cheek meat 1% of the time and that when such contamination occurs, it amounts to 1/1000 of the infectivity in TG.

3.2 Impact of Alternative Assumptions on Cattle Infected and Human BSE Exposure

We used sensitivity analysis to identify the most important sources of uncertainty. In particular, we evaluated the extent to which each assumption or parameter individually influences model predictions for two cumulative outcomes over a 20-year period – the total number of cattle that become infected after the introduction of 10 infected animals at the beginning of the period, and the amount of BSE infectivity (quantified in terms of the number of cattle oral ID_{50}s) in food available for human consumption over that period. The purpose of the sensitivity analysis is to identify assumptions that should be regarded as candidates for further refinement.

To conduct the sensitivity analysis, we first ran the base case simulation 5,000 times and recorded the distribution of outcomes for each of the two outcomes of interest (total additional cattle infected, and total potential human exposure to BSE infectivity). To evaluate the contribution of an individual parameter or assumption to the uncertainty of these two outcomes, we altered each assumption, one at a time, setting all other assumptions to their base case values. In particular, each assumption was individually set equal to its "worst case" bounding value. The "worst case" value refers to that bounding value for an assumption expected to result in the

largest predicted risk of BSE spreading. For each alternative value, we again ran the simulation 5,000 times and recorded the distribution of values for the two outcomes described above[4].

Typically, sensitivity analysis also involves estimation of risk for each parameter's best case value, as well as for each parameter's worst case value. For this project, we determined that evaluation of the best case values was not necessary because use of base case assumptions yields a disease reproductive rate constant for BSE in the U.S. (R_0) substantially less than unity. That is, the model predicts that the prevalence of BSE decreases over time and eventually reaches zero with near certainty (see Section 4.1). Using more optimistic assumptions for a parameter would only result in a prediction that the spread of BSE is even more limited than the base case suggests. Although such a finding would differ quantitatively from the base case, further analysis is not important because it would not qualitatively alter our conclusions.

The worst case bounding assumptions for each parameter reflect the judgment of this report's authors given the available scientific literature. Although these assumed values are not intended to represent absolute bounds on a parameter's value, we have selected them with the intention of identifying levels beyond which a parameter's true value is very unlikely to fall. The difference between the simulation results produced using a parameter's base case assumption and that parameter's worst case bounding assumption represents the maximum extent to which that parameter's uncertainty can independently increase the predicted spread of BSE.

Instead of conducting deterministic sensitivity analyses for each parameter (*i.e.*, comparing the simulation results corresponding to the base case to the simulation results corresponding to each parameter's bounding case), we could have conducted a probabilistic sensitivity analysis that would characterize the distribution of results corresponding to the uncertainty associated with each parameter. However, conducting probabilistic analyses would require specification of a probability distribution for each uncertain parameter over its range of plausible values, rather than merely identifying the bounds on this range. It is our judgment that the available information is inadequate to develop the needed probability distributions. The task is complicated because many of the parameters are not individual numbers but are instead

[4] One combination of worst case parameters (see Section 4.2.1) was run 780 times because the number of predicted animals infected was often so large that the simulation ran very slowly. The 780 runs required approximately 250 hours of run time on a 3 GHz Windows-compatible computer. Although the limited number of runs reduced the precision of the results, the results were adequately precise for the purpose of

collections of values. Consider, for example, the three multinomial distribution that specifies the probability of a mis-split, the use of AMR, and the removal of the spinal cord (see Section 3.1.2.7) during the slaughter process.

In addition to conducting a univariate sensitivity analysis, we also evaluate the synergistic impact of assigning worst case values to multiple sets of simulation parameters simultaneously. An alternative approach would be to simultaneously sample values from distributions for each assumption. However, as noted earlier, development of meaningful distributions for each assumption is not practical given the information that is available.

Because the BSE simulation model has a large number of parameters and assumptions, and because evaluating each set of assumptions takes a substantial amount of time (in particular, one set of 5,000 runs takes around nine hours on a 1.8 GHz Intel Pentium IV Processor), we first eliminated from consideration those parameters for which such analysis is not necessary. The remainder of Section 3.2 describes this step in further detail. We identify bounds for those parameters and assumptions not eliminated from further consideration. Qualitative arguments for eliminating a parameter from further consideration fall into three categories.

First, if the base case results indicate that a set of factors makes only a minor contribution to either of the key simulation model outcomes, then modifying assumptions related to those factors is unlikely to influence the results substantially. For example, assumptions related to blood meal can be eliminated from further consideration because blood meal represents a very limited portion of the infectivity to which cattle are exposed. The relative importance of blood as a contributor to infection can be quantified by comparing the mean number of new cases arising from this mode transmission (0.006) to the mean total for the number of infected animals excluding those imported at the beginning of the simulation (4.3) (see Table in Section 1 of Appendix 3A). Because the simulation was run 5,000 times, an average of 0.006 indicates that over all 5,000 simulation runs, a total of thirty cattle became infected as a result of exposure to blood meal. That contribution is roughly 0.1% of the total number of animals infected excluding imports over all 5,000 simulations (21,500).

determining that this combination of worst case assumptions often results in conditions that cause BSE to spread, rather than to die out.

Second, a parameter can be eliminated from consideration if its range of plausible values and the extent to which changing its value influences the results suggest that the overall impact will be minimal. For example, as discussed in Section 3.2.1.1, because changing the assumed size of the cattle population should have virtually no effect on the simulation results, using the simulation model to evaluate that impact is not necessary.

Finally, if the relationship between a parameter's value and the simulation results is well-understood, then it is not necessary to use the simulation model to quantify this relationship. For example, changing the proportion of cattle brains consumed by humans has virtually no impact on the number of infected cattle for any plausible (*i.e.*, relatively small) value for this parameter. Moreover, it is clear that doubling this parameter, for example, will double potential human exposure to BSE from consumption of brains.

The remainder of Section 3.2 reviews the Harvard model's parameters and assumptions, following the order in which the parameters were presented in Section 3.1. Section 2 in Appendix 2 to this report details the parameter file changes made for each of these scenarios.

3.2.1 Cattle Population Dynamics

3.2.1.1 Size of Cattle Population, Birth Rates, Slaughter Rates, and Rates of Death Due to Other Causes

None of these parameters in this category need to be evaluated quantitatively using the simulation model. First the values for all of these parameters are based on empirical data and direct observation. We therefore believe it is unlikely that they are incorrect by even as much as a factor of two. It is our judgment that in most cases, the error affecting any of these parameters is substantially less than this amount.

As noted in Section 3.1.1.1, the assumed size of the cattle population size (140 million) is 40% larger than the known value for this parameter (around 100 million). However, this parameter has virtually no impact on the simulation. In particular, the number of animals that become infected at each time step in the simulation depends on the number of animals infected at the preceding time step, along with the magnitude of any assumed exogenous introduction of BSE infectivity. Neither of these factors depends on the size of the cattle population unless the number of infected animals becomes an appreciable fraction of the total number of animals. In

particular, a very small assumed population size would appreciably increase the probability that recycled material contaminated with BSE would be consumed by an animal already infected with BSE, thus decreasing the simulated rate at which the disease would spread among the cattle population. Because the total number of infected animals in any of the scenarios tested is always at least several orders of magnitude less than the size of the cattle population, changing the assumed cattle population size would have virtually no impact on the simulation results.

3.2.1.2 Proportion of Animals That Die on Farm That Are Rendered

The worst case scenario assumes that this proportion is 99%.

3.2.1.3 Cattle Consumption of Bypass Protein and Blood Meal

Because the protein and blood meal consumption rates are used to probabilistically allocate infected material among different type/gender/age cattle groups, the absolute magnitudes of these values do not matter. In particular, multiplying all the consumption rates by a constant would not affect the simulation results. However, changing the relative magnitudes of these consumption rates would influence the extent to which cattle in different age groups are exposed to contaminated feed and blood meal.

Because blood meal is a minor contributor to the spread of BSE (see introduction to Section 3.2), the assumed relative consumption rate for blood meal is not important. On the other hand, protein consumption is important because it accounts for the vast majority of the new BSE infections in the base case. The base case assumes that the calf daily protein consumption rate is more than half as great as the corresponding rate for older cattle. Because susceptibility to infection is greater among young animals than it is among older animals, shifting more of the consumption to calves would increase the rate at which BSE spreads. However, it is unlikely that the adult bypass protein consumption rate is substantially smaller than we have assumed relative to calf bypass protein consumption rate. In particular, adult dairy cows must consume bypass protein to maintain high milk production rates. Because it is unlikely that young cattle are consuming a substantially greater proportion of the bypass protein than we have assumed, we conclude that it is unnecessary to evaluate this issue quantitatively.

3.2.1.4 BSE Dose Response

Recall that the dose-response assumptions fall into two categories – the shape of the dose-response relationship (*e.g.*, linear or threshold), and the relationship between age and relative susceptibility to the BSE agent (*i.e.*, older animals are far less likely to become infected than younger animals exposed to BSE to the same degree).

Shape of the dose-response relationship: The base case assumes the dose-response relationship does not have a threshold. If we had assumed a threshold, the number of additional BSE cases predicted by the simulation would, if anything, have been smaller. There is no biologically plausible basis for assuming a super-linear dose response relationship. Hence, the base case assumption must be regarded as either neutral or conservative. For reasons described in the introduction to Section 3.2, quantitative evaluation of this assumption is therefore not necessary.

Relative susceptibility *vs.* age: The assumption that susceptibility to infection decreases with age is based on both biological considerations (Section 2.1.3) and statistical modeling of the UK BSE outbreak (Section 3.1.1.4 and Appendix 1, Section 2.3.4). It is therefore unlikely that susceptibility is independent of age or that it increases with age. However, it is possible that susceptibility decreases more slowly with respect to age than is assumed in our base case. For the purpose of evaluating the impact of this assumption on the model's predictions, our worst case assumes that susceptibility falls approximately half as fast (40% per year) as we assume in the base case (85% per year), and that as the animal ages, susceptibility asymptotically approaches 10% of its value at birth (same as in base case).

3.2.1.5 Maternal Transmission

Maternal transmission is a relatively unimportant mode of transmission in the base case. Of the approximately 21,500 animals that became infected (excluding imports) during the 5,000 base case simulation runs, 3,200 (15%) were infected due to maternal transmission. It is therefore unlikely that other plausible values for the parameters influencing maternal transmission would result in an important number of infections *via* maternal transmission. We therefore do not quantitatively evaluate the impact of alternative values for assumptions related to this mode of transmission.

3.2.1.6 BSE Incubation Period, and Time Until Death Following Onset of Clinical Signs

BSE incubation period: The incubation period for BSE (*i.e.*, the time between exposure and development of clinical signs) is somewhat uncertain because the time at which exposure leading to infection occurs is not known precisely. On the other hand, typical values for this parameter are relatively well understood because the data from the UK outbreak have been extensively analyzed, *e.g.*, (Ferguson 1997b; Donnelly 2000). A shorter incubation period could increase the predicted rate at which BSE spreads because it would increase the probability that an animal would reach the later stages of infection, when infectivity loads in the animal climb sharply, prior to being sent to slaughter. To quantify the potential impact of this assumption's uncertainty, the worst case assumes that the incubation period distribution is a factor of two less than the base case (*i.e.*, all percentiles of the distribution equal one-half their base case values).

Time until death following the onset of clinical signs: The duration between the onset of clinical symptoms and death from BSE is well understood because these two events can be directly observed. The uncertainty associated with this assumption is therefore unlikely to be substantial. Moreover, it is unlikely that changing this assumption would substantially affect the simulation's predictions. Once an animal develops clinical signs, we assume that its infectivity load remains unchanged (10,000 ID_{50}s in the base case). Decreasing the assumed duration between the onset of signs and death would tend to increase the predicted rate at which BSE spreads because fewer of the animals that develop clinical signs would be sent to slaughter prior to dying from BSE. The base case assumes that 90% of the clinical animals sent to slaughter would be detected and disposed of in a manner that could not expose any additional animals to the BSE agent. Among those animals that die on the farm (for whatever reason) prior to being sent to slaughter, the base case assumes that only 15% are disposed of in a manner that cannot lead to the exposure of any additional animals to BSE. For the purpose of evaluating the sensitivity of the simulation results to the assumed duration between the development of clinical BSE signs and death, we assume that for the worst case, this duration is uniformly distributed between 1 and 4 months.

3.2.2 The Slaughter Process

3.2.2.1 Level of Infectivity and Distribution of Infectivity Throughout the Carcass

Total infectivity in carcass: Because the assumption that 0.1 g of brain from a clinically affected animal amounts to one ID_{50} is thought to be conservative (Section 4.2 in (DNV (Det Norske Veritas) 1997)), it is unlikely that the amount of infectivity in a single animal substantially exceeds our base case estimate of 10,000 ID_{50}s. In fact, the latest pathogenesis results indicate that 0.67 g of tissue from a full blown BSE case represents one ID_{50} (confidence interval of 0.24g to 1.83 g) (p. 83 in (Vossen 2003)). To address the possibility that continuing experiments will find that smaller amounts of CNS tissue from clinically affected animals can result in the transmission of BSE, our worst case assumes that the total infectivity in a clinically affected animal is as much as 20,000 ID_{50}s.

Distribution of infectivity throughout the carcass: We consider two deviations from the base case. First, because some experiments have shown that other TSEs can be present in blood (European Union Scientific Steering Committee 2000e), we consider the possibility that 0.016% of the infectivity in an animal with BSE is carried in blood. This level of infectivity is consistent with the assumption that its concentration is at the level of detection in an animal with a full-blown case of BSE. Our assumptions reflect the judgment of the SSC that one kg of any cattle tissue negative for infectivity in the mouse bioassay could contain as much as ten oral cattle ID_{50}s (Annex 2 in (European Union Scientific Steering Committee 2000a)). Hence, we assume that the 3.8 kg of blood dried blood that can be recovered from an average steer (Romans 1974) could contain 38 cattle oral ID_{50}s.

Second, we consider the possibility that the amount of infectivity in trigeminal ganglia is higher than the level assumed in the base case. The base case assumes that the trigeminal ganglia harbor no infectivity until the animal reaches the halfway point between exposure to BSE and manifestation of clinical signs, after which time, we assume the trigeminal ganglia harbor 2.6% of the total infectivity. The base case further assumes that no trigeminal ganglia tissue is recovered for human consumption. The sensitivity analysis evaluates the impact of assuming that 0.1% of the infectivity in the trigeminal ganglia (as much as 0.0026% of the total infectivity) is recovered for potential human consumption with 1% probability, and that with 99% probability, none of this infectivity is recovered for human consumption.

3.2.2.2 Antemortem Inspection

The worst case scenario assumes that cattle with clinical signs of BSE will be detected at antemortem inspection with 50% probability (compared to 90% probability for the base case).

3.2.2.3 Stunning

The prevalence of pneumatic stunning devices appears to be zero in the U.S. Even in the late 1980s, before use of this technology fell out of favor, the proportion of animals stunned using pneumatic devices was only 15% (see Section 3.1.2.3). For the purpose of evaluating this assumption's impact on the simulation results, the worst case scenario assumes that the current prevalence also is 15%.

Both the malfunction probability and the production of emboli have been measured empirically and are unlikely to be substantially greater than assumed by the base case scenario. Because the worst case scenario assumption for the prevalence of pneumatic stunners is so conservative, and because device malfunction and emboli production have been empirically measured, it is not necessary to consider more conservative assumptions for these last two parameters.

3.2.2.4 Exsanguination

The use of the pneumatic stunner increases the probability that blood gathered by exsanguinations will be contaminated with brain tissue from zero to 30%. As noted in Section 3.2.2.3, the worst case scenario assumption for the prevalence of pneumatic stunners is very conservative. The worst case scenario for exsanguinations assumes the same elevated prevalence for pneumatic stunners of 15%. It also assumes that blood gathered under these circumstances will be contaminated with 100% probability.

3.2.2.5 Disposition of Tissues and Potential for Human Exposure

Table 3-8 details the base case and worst case assumptions regarding the proportion of tissue recovered for human consumption. Note that none of these sets of assumptions reflect a specified risk material (SRM) ban.

Table 3-8
Base Case and Worst Case Values for the Proportion of Tissues Recovered from Cattle for Human Consumption

Tissue	Base Case	Worst Case
AMR Meat	0.98	0.98
Blood	0.05	0.3
Bone (in-bone cuts of meat)	0.98	0.98
Brain	0.01	0.02
Dorsal root ganglia	0	0
Eyes	0.001	0.002
Ileum	0.01	0.02
Heart	0.5	0.6
Kidney	0.25	0.35
Liver	0.6	0.7
Lung	0	0
Muscle	0.98	0.98
Spinal Cord	0.01	0.02
Trigeminal ganglia	0	0

3.2.2.6 Splitting and Aerosolization

The base case results indicate that aerosolization is responsible for less than 1% of potential human exposure. Moreover, the aerosolization assumptions, which are based on empirical measurements (see Section 3.1.2.6) are probably conservative because we assume steam washing of the carcass has no effect on the amount of deposited contamination. Because the contribution of aerosolization to potential human exposure appears limited even with these conservative assumptions (contaminated muscle meet in the base case accounts for 0.099 ID_{50}s on average, compared to a total of 39 ID_{50}s available for potential human consumption), it is unnecessary to analyze its contribution to uncertainty quantitatively.

3.2.2.7 Disposition of the Spinal Cord and Dorsal Route Ganglia

The base case results indicate that contamination of AMR meat accounts for more than half of all potential human exposure to BSE. Because of its apparent importance, we use quantitative sensitivity analysis to evaluate the impact of uncertainty for this set of parameters on the simulation model's predictions.

The assumed proportion of animals processed using AMR is already relatively high for older animals (65% for animals 12 to 23 months, and 60% for animals 24 months or older). Our

worst case scenario reduces the proportion of animals whose spinal cords are removed from 50% to 10% when AMR is not used, and from 98% to 80% when AMR is used. Finally, the worst case scenario doubles the assumed base case mis-split frequency from 5% to 10% for animals below the age of 24 months, and from 8% to 16% among animals 24 months or older.

3.2.2.8 Postmortem Inspection

The base case results indicate that almost all human exposure is due to consumption of AMR (51%), brain (24%), beef on bone (12%), and spinal cord (9%). The contamination in AMR meat derives from both spinal cord and DRG. The proportion of these tissues passing post mortem inspection is already very high (90% for brain, spinal cord, and DRG, and 98% for beef on bone). Hence, increasing the assumed proportion of tissue passing post mortem inspection would have at most a limited effect on predicted human exposure.

3.2.2.9 Recovery of Material for Potential Human Consumption

For material passing post mortem inspection, almost all AMR meat and all beef on bone are recovered for potential human consumption (98% in both cases). Although the base case scenario assumes that only 1% of all brains and spinal cords are recovered for human consumption, it is unlikely that consumption is substantially higher than we have assumed for either of these tissues. In any case, it is unnecessary to use the simulation model to quantify the impact of alternative assumptions for the food use recovery parameters because the impact on human exposure would be linear (*e.g.*, doubling the proportion of brains recovered for human consumption would double potential human exposure attributable to brain consumption from the base case value of 9.2 ID_{50}s to 18.4 ID_{50}s).

Nor would any plausible change in the proportion of tissue recovered for potential human consumption substantially affect the predicted spread of BSE among cattle. Any material diverted away from potential human consumption would be rendered. The base case predicts that rendering eliminates 37,000 ID_{50}s, which means that the total amount of infectivity sent to rendering must exceed 37,000 ID_{50}s . The total amount of infectivity in material recovered for potential human consumption is approximately 0.1% of this total, so diverting this material to rendering would have virtually no effect on the predicted spread of BSE.

3.2.3 Rendering and Feed Production

For assumptions related to the rendering process, the feed production process, and to on-farm feed practices that have been evaluated as part of the uncertainty analysis, Table 3-9 details base case and worst case values.

Table 3-9
Base Case and Worst Case Values for Render Process, Feed Production Process, and On-Farm Feed Practice Assumptions

Assumption	Base Case	Worst Case
Proportion of animals rendered using various technologies		
Batch (3.1 logs reduction)	5%	5%
Continuous/fat added (2.0 log reductions)	45%	20%
Continuous/no fat added (1.0 log reduction)	45%	70%
Vacuum (no reduction)	5%	5%
Rendering – Contamination of non prohibited MBM by prohibited MBM in mixed facilities		
Probability for a particular prohibited packet	14%	25%
Magnitude of contamination[a]	0.1%	1.0%
Rendering – Probability that prohibited MBM will be mislabeled as non-prohibited MBM when produced by either a mixed or prohibited rendering plant	5%	10%
Feed Production – Contamination of non prohibited MBM by prohibited MBM in mixed facilities		
Probability for a particular prohibited packet	16%	16%
Magnitude of contamination[a]	0.1%	1.0%
Feed Production – Probability that prohibited feed will be mislabeled as non-prohibited feed when produced by either a mixed feed production plant	5%	33%
Probability that correctly labeled prohibited feed will be incorrectly administered to cattle	1.6%	15%

Notes:

a. Refers to the proportion of the prohibited packet that ends up in the non-prohibited packet when contamination occurs.

3.2.4 Potential Human Exposure

Section 3.2.1.9 discusses the worst case assumptions for this set of parameters.

3.2.5 Simultaneous Evaluation of Multiple Worst Case Assumptions

As described in the introduction to Section 3.2, we report results for simulations that simultaneously assign worst case values to multiple parameters. The sets considered simultaneously are the cattle population dynamic assumptions (base case values described in Section 3.1.1), the slaughter process assumptions (base case values described in Section 3.1.2), and the MBM production, feed production, and feed practice assumptions (base case values described in Section 3.1.3). We also evaluate the impact of simultaneously assigning all assumptions to their worst case in each possible pair of these sets (cattle population dynamics and slaughter process; cattle population dynamics and MBM production, feed production, and feed practices; and slaughter process and MBM production, feed production, and feed practices).

3.2.6 Summary

Table 3-10 summarizes the worst-case simulations conducted and identifies reasons for using the simulation model to quantify the impact of other worst case assumptions.

Table 3-10
Summary of Worst Case Analyses

			Worst Case Not Simulated Because		
Section	**Description**	**Worst Case Simulated**	**Base Case Contribution Small**	**Parameter's Uncertainty and/or Impact on Output Small**	**Relationship Between Parameter and Simulation Output Well Understood**
Cattle Population Dynamics					
3.2.1.1	Cattle population parameters			X	
3.2.1.2	Die on farm render rate	X			
3.2.1.3a	Bypass protein consumption			X	
3.2.1.3b	blood consumption		X		
3.2.1.4a	BSE dose response shape			X	

Table 3-10
Summary of Worst Case Analyses

Section	Description	Worst Case Simulated	Worst Case Not Simulated Because		
			Base Case Contribution Small	Parameter's Uncertainty and/or Impact on Output Small	Relationship Between Parameter and Simulation Output Well Understood
3.2.1.4b	BSE susceptibility	X			
3.2.1.5	Maternal transmission		X		
3.2.1.6	BSE incubation and time until death	X			
Slaughter Process					
3.2.2.1a	Total infectivity in carcass	X			
3.2.2.1b	BSE in blood	X			
3.2.2.1c	BSE in trigeminal ganglia	X			
3.2.2.2	Antemortem inspection	X			
3.2.2.3a	Prevalence of pneumatic stunners	X			
3.2.2.3b	Stunner malfunction rate			X	
3.2.2.3c	Emboli formation rate			X	
3.2.2.4	Exsanguination	X			
3.2.2.5	Disposition of tissues for human consumption	X			
3.2.2.6	Splitting		X		
3.2.2.7	Spinal cord and DRG disposition	X			

Table 3-10
Summary of Worst Case Analyses

Section	Description	Worst Case Simulated	Worst Case Not Simulated Because		
			Base Case Contribution Small	Parameter's Uncertainty and/or Impact on Output Small	Relationship Between Parameter and Simulation Output Well Understood
3.2.2.8	Postmortem inspection			X	
3.2.2.9	Recovery of material for human consumption			X	X
MBM Production, Feed Production, and Feeding Practices					
3.2.3.1	Render technology	X			
3.2.3.2	MBM contamination	X			
3.2.3.3	MBM mislabeling	X			
3.2.3.4	Feed contamination	X			
3.2.3.5	Feed mislabeling	X			
3.2.3.6.	Misfeeding	X			

3.3 The Base Case: Impact of Alternative Sources of Infectivity

This section describes how we evaluated the impact of different sources of infectivity on the model's predictions. For each of the scenarios considered, we adopted the base case assumptions. Infectivity sources evaluated include: spontaneous development of disease (Section 3.3.1), import of infected cattle or contaminated feed (Section 3.3.2), scrapie in sheep (Section 3.3.3), chronic wasting disease (CWD) in deer/elk-derived protein supplements (Section 3.3.4), CWD from direct contact with infected mule deer, white tail deer and/or elk (Section 3.3.5), transmissible mink encephalopathy in mink (Section 3.3.6), a TSE in pigs (Section 3.3.7), a TSE in chickens (Section 3.3.8), and recycled waste (Section 3.3.9). Section 3 in Appendix 2 to this report details the parameter file changes made for each of these scenarios.

3.3.1 Spontaneous BSE

Because there are no measurements of an incidence rate for spontaneous BSE in cattle, we use the observed age-specific sporadic rate for CJD in humans as a proxy, adjusting the ages to reflect the difference between the natural lifespan for bovines (approximately 20 years) (Nowak 1983) and the much longer natural lifespan for humans (approximately 75 years). For example, the CJD incidence rate for 75-year old humans is assumed to represent the BSE incidence rate for 20-year old bovines. Making this adjustment and taking into account the incubation period for BSE yields the age-specific rates that appear in Table 3-11.

Developing age-specific rates is necessary because the often-quoted incidence rate for sporadic CJD in humans of one per million per year hides substantial variation across age groups. The disease is virtually never seen before age 30 and has a peak incidence between ages 60 and 65 (Collinge 1997). If this pattern (Figure 3-8) is applicable to sporadic BSE, then estimated rates in cattle must reflect the age structure of the disease.

Finally, recall that the human CJD rates used to estimate the incidence rate for BSE represent the rate at which clinical cases appear in the population. Therefore, the rates in Figure 3-8 cannot be used to estimate the rate at which new pre-clinical cases might develop. Instead, the case age-specific clinical incidence rates must be advanced by the duration of the period between infection and the manifestation of clinical signs. The median incubation period for BSE is approximately four years (Section 3.1.1.6). Table 3-11 lists the sporadic BSE rates inferred from the human sporadic CJD rates. Note that the fourth column from the left (the spontaneous BSE new infection rate) is similar to the third column from the left (the spontaneous CJD new clinical case rate) but is offset by three rows, representing four years.

Table 3-11
Human Age Categories, Equivalent Cattle Age Categories, and Age-Specific Human Sporadic CJD Rates[a]

Human Age Category (years)	Equivalent Cattle Age Category (years)	Annual Clinical CJD Incidence Rate (per million)	Annual Pre-Clinical BSE Incidence Rate (per million)
0 to 4	0.0 to 1.0	<0.01	0[b]
5 to 9	1.3 to 2.4	0	< 0.01
10 to 14	2.6 to 3.7	0	<0.01
15 to 19	4.0 to 5.0	0	0.04
20 to 24	5.3 to 6.4	<0.01	0.08
25 to 29	6.6 to 7.7	<0.01	0.16
30 to 34	8.0 to 9.07	0.04	0.45
35 to 39	9.3 to 10.4	0.08	0.99
40 to 44	10.6 to11.7	0.16	2.14
45 to 49	12.0 to13.0	0.45	3.55
50 to 54	13.3 to14.4	0.99	5.03
55 to 59	14.6 to15.7	2.14	5.75
60 to 64	16.0 to17.0	3.55	5.6
65 to 69	17.3 to18.4	5.03	3.94
70 to 74	18.6 to19.7	5.75	2.42
75 to 79	20.0 to21.0	5.6	2.42[c]
80 to 84	21.3 to22.4	3.94	2.42[c]
85 +	22.6 +	2.42	2.42[c]

Notes:

a. Adapted from (Holman 1995; anonymous 1996)

b. We assume that the spontaneous CJD case observed in a young child was spongiform degeneration of infancy erroneously coded as CJD.

c. We assume that the spontaneous rate for BSE remains unchanged at ages beyond what can be inferred from the corresponding spontaneous CJD data.

3.3.2 Import of Infected Cattle or Contaminated Cattle Feed

Because APHIS has banned the import of cattle and feed from countries in which the presence of native BSE has been documented (see Section 2.3.2), the import of even a single infected animal is not highly likely. Given that even in the UK, the prevalence of BSE is relatively small, it is implausible that a large number of animals infected with BSE might be imported into the United States.

Nonetheless, in order to evaluate the robustness of the U.S. cattle population against the introduction of BSE-infected animals, we have simulated the introduction of 1, 5, 20, 50, 200,

and 500 infected animals into the U.S. We assume that the imported animals are 12-month old female dairy cattle that have just become infected. The number of animals was chosen arbitrarily to bound any potential introduction. Dairy cows were modeled to increase the likelihood that animals would survive long enough to potentially introduce significant infectivity into the U.S. system.

In order to evaluate the robustness of the U.S. cattle population against the introduction of contaminated feed, we have simulated the introduction of feed containing 10,000 ID_{50}s. Because rendering reduces the amount of infectivity by an average of a factor of approximately ten, this scenario is equivalent to the import of feed contaminated with the remains of ten full blown cases of BSE. We also assume that this feed is very widely distributed so that no single animal receives more 0.2 ID_{50}s. As a result, this scenario does not "waste" any of the infective agent by sending more to any single animal than is needed to guarantee that animal's infection.

3.3.3 Domestic Scrapie

The transmission of scrapie from sheep to cattle is one of the primary hypotheses for the origin of BSE (Horn 2001). Moreover, scrapie is present in the United States. Although no North American strain of scrapie has been successfully transmitted to cattle exposed orally to the agent (Cutlip 2001), we evaluate the impact of assuming that such transmission is possible. In particular, if transmission is possible, we estimate that the rendering of scrapie-infected sheep could expose the U.S. cattle population to one cattle oral ID_{50} in feed each month. The derivation of this estimate is based on the assumption that the number of cattle oral ID_{50}s administered to cattle is equal to the product of 1) the number of scrapie-infected sheep rendered each year, 2) the number of sheep oral ID_{50}s per infected animal, 3) the inverse of the cattle-sheep species barrier, and 4) the proportion of infectivity sent to rendering that survives rendering and is ultimately administered to cattle.

Number of scrapie-infected sheep rendered: We estimate the number of clinical sheep and the number of pre-clinical sheep separately. A total of approximately 180,000 federally inspected mature sheep are slaughtered each year in the U.S. (U.S. Department of Agriculture 1998). Assuming that the prevalence of scrapie is the same in the U.S. as it is in the UK (11%) (Simmons 2000), there are approximately 20,000 sheep with scrapie slaughtered each year. Although sheep with clinical signs would ordinarily be detected at AM inspection and directed to

incineration, we assume that they are rendered. We assume that 1% of the infected sheep fall into this category, suggesting that approximately 200 sheep with clinical signs are slaughtered each year. Note that these estimates are likely to be conservative because the true prevalence of scrapie in the U.S. is probably less than it is in the UK.

Number of sheep oral ID_{50}s per clinical scrapie case: For sheep with clinical scrapie, we assume that this quantity is 10,000, *i.e.*, the same as the number of cattle oral ID_{50}s per clinical case of BSE. In order to estimate the amount of infectivity in sheep that are not yet showing clinical signs of disease, we assume that the infectivity load for scrapie follows the same temporal pattern as BSE does in cattle (see Figure 3-6). As a result, we estimate the average number of ID_{50}s in a sheep to equal the time-averaged ID_{50} burden in cattle over the period prior to the development of clinical signs. This average is approximately 600 ID_{50}s.

The cattle-sheep species barrier: We assume that the species barrier between sheep and cattle is 1,000, *i.e.*, 1 sheep oral ID_{50} is equivalent to 0.001 cattle oral ID_{50}s. This assumption is based on an evaluation of the relative transmissibilty of BSE from cattle to mice (Bradley 1999) and on results from *in vitro* conversion studies (Raymond 1997). The true species barrier is unknown and may be substantially higher. For example, no North American strain of scrapie has been successfully transmitted to cattle exposed orally to the agent (Cutlip 2001).

Proportion of infectivity surviving rendering and administered to cattle: We used the simulation model, along with the base case assumptions to estimate the proportion of infectivity in prohibited material that is ultimately administered to cattle. In particular, we repeatedly simulated the rendering of a bovine with 1 ID_{50} using the base case assumptions. Based on 1,000 runs of this simulation, we estimate that under the conditions described the base case, the average number of ID_{50}s administered to cattle amounted to 8×10^{-4}, *i.e.*, a little less than 0.1% of the infectivity in prohibited material survives rendering and is ultimately administered to cattle.

Total infectivity to cattle: For sheep with clinical scrapie, the product of the four quantities just described is approximately 2 cattle oral ID_{50}s per year. For sheep not yet showing clinical signs of disease, the product is approximately 10 cattle oral ID_{50}s per year. The total amounts to 12 cattle oral ID_{50}s annually, or 1 cattle oral ID_{50} per month. Note that this estimate probably overstates actual cattle exposure for two reasons. First, it is likely that the true species

barrier is greater than the value of 1,000 used here. Second, the prevalence of scrapie in the U.S. is probably less than the UK prevalence rates adopted here.

3.3.4 Chronic Wasting Disease: Oral Exposure

The FDA feed ban prohibits the use of rendered material derived from cervids in the production of feed to be administered to cattle. However, because the ban is not completely effective, cattle may be exposed to cervid-derived protein and hence to CWD. This section describes our estimate of an upper bound on this exposure. Annual cattle exposure to CWD attributable to consumption of cervid-derived protein is the product of the 1) number of diseased animals harvested, 2) the number of cervid ID_{50}s per slaughtered case, 3) the fraction of animals rendered, 4) the inverse of the species barrier, and 5) the proportion of infectivity surviving rendering and administered to cattle. As described below, we estimate that present-day exposures to CWD among the U.S. cattle population could be as high as two cattle oral ID_{50}s per year, although the true value is likely to be substantially lower and could be zero.

Number of diseased animals harvested: Table 3-12 details the disease prevalence rate, population size, and annual harvest rate for the three species suspected of harboring CWD. In one respect, these estimates are likely to overstate the true values because they assume the prevalence rate for the endemic area applies to the entire population. On the other hand, because monitoring of CWD is limited to post mortem evaluation of brain tissue, it is possible that surveillance fails to detect animals with less advanced disease.

Table 3-12
Annual Number of CWD-Infected Animals Harvested

Species	Disease Prevalence[a]	Population Size[b]	Annual Harvest Rate[b]	Infected Animals Harvested per Year[c]
Mule deer	4.9%	2×10^6	20-25%	24,500
White tail deer	2.1%	3.2×10^7	25%	168,000
Rocky Mountain Elk	0.5%	1.0×10^6	15-20%	1,000
Total				194,000

Notes:

a. Refers to the estimated prevalence of CWD among animals harvested from the CWD endemic areas of north central Colorado and southeastern Wyoming (Miller 2000).

b. Source (Rocky Mountain Elk Foundation 1997); Lloyd Floyd, personal communication; Quality Deer Management Association's.

c. Computed using the upper bound annual harvest rate in the fourth column from the left.

Number of cervid ID_{50}s per case: Because the prevalence rate has been estimated on the basis of post mortem evaluation of brain tissue, they may reflect only those animals that have advanced disease. We assume that there are 10,000 cervid oral ID_{50}s per case of disease.

Fraction of animals rendered: Only a small portion of cervids harvested for human consumption are likely to be rendered at all. Those that are rendered are most often processed by an independent facility that handles only prohibited rendered material (Franco 2001). We assume that 10% of the harvested cervids are rendered.

The species barrier: As noted in Section 2.3.4, the species barrier for the transmission of CWD from cervids to cattle appears to be between 10^5 and 10^{12}. We conservatively assume that the species barrier value is 10^5.

Proportion of infectivity surviving rendering and administered to cattle: As described in Section 3.3.3, under present-day conditions (*i.e.*, with the adoption of the feed ban), total cattle population exposure to infectivity is approximately 0.1% as great as the amount of infectivity in animals sent to rendering.

Total Cattle Population Exposure: Under present-day conditions, total exposure to CWD is estimated to amount to no more than 2 cattle oral ID_{50}s per year, or approximately 0.2 cattle

oral ID_{50}s per month. As noted above, this estimates reflects several assumptions that are potentially very conservative. The true level of exposure is perhaps much lower.

3.3.5 Chronic Wasting Disease: Lateral Transmission

Because the potential impact of this source is insignificant (see Section 2.3.5), we do not quantitatively model its impact on the prevalence of BSE in the U.S. cattle population or its contribution to contamination of the U.S. food supply.

3.3.6 Mink

As is the case with cervids, FDA regulations prohibit the administration to cattle of feed fortified with protein derived from mink, although this ban may not completely prevent such exposures. This section describes our development of an upper bound estimate on this exposure, which we estimate to be on the order of 1 cattle oral ID_{50} annually. The true value is likely to be substantially lower, and could be zero. Our methodology is similar to that used to evaluate the exposure risk associated with CWD. Annual cattle exposure to TME attributable to consumption of mink-derived protein is the product of the 1) number of diseased animals harvested, 2) the number of mink ID_{50}s per animal slaughtered, 3) the fraction of animals rendered, 4) the inverse of the species barrier, and 5) the proportion of infectivity surviving rendering and administered to cattle.

Number of diseased animals harvested: A total of 2.6 million mink are harvested in the U.S. annually (U.S. Department of Agriculture 2001b). The prevalence of disease is unknown. We assume that the prevalence of clinical and pre-clinical disease are both similar to the corresponding rates for scrapie, or approximately 0.1% and 10%, respectively. Hence, we estimate that there are 2,600 clinical animals and 260,000 pre-clinical animals slaughtered each year.

Number of mink ID_{50}s per case: As we estimated for scrapie, we assume that pre-clinical animals harbor an average of 600 mink ID_{50}s, whereas clinical animals harbor 10,000 mink ID_{50}s.

Fraction of animals rendered: We estimate that 60% of slaughtered mink are rendered (Platt 2001).

The species barrier: Experimental transmission of TME from the Stetsonville outbreak to cattle via i.c. inoculation resulted in animals developing a fatal spongiform encephalopathy (Marsh 1991), although it appeared to be distinct from BSE. As with CWD, we assume that the species barrier for TME transmitted to cattle is 10^5.

Proportion of infectivity surviving rendering and administered to cattle: As in the case of CWD, we assume that this value is now 0.1%.

Total infectivity reaching cattle: Total infectivity reaching cattle from clinical TME cases amounts to 0.2 cattle oral ID_{50}s annually, while the corresponding value for pre-clinical animals is 0.9 cattle oral ID_{50}s. The total amounts to 1 cattle oral ID_{50} per year, or approximately 0.1 cattle oral ID_{50}s per month. Because this source exposes cattle to substantially less infectivity than does scrapie (as modeled in Section 3.3.3), we do not quantitatively model its impact on the prevalence of BSE in the U.S. cattle population or its contribution to contamination of the U.S. food supply.

3.3.7 Pigs

Because the potential impact of this source is insignificant (see Section 2.3.7), we do not quantitatively model its impact on the prevalence of BSE in the U.S. cattle population or its contribution to contamination of the U.S. food supply.

3.3.8 Poultry

Because the potential impact of this source is insignificant (see Section 2.3.8), we do not quantitatively model its impact on the prevalence of BSE in the U.S. cattle population or its contribution to contamination of the U.S. food supply.

3.3.9 Recycled Waste

Because the potential impact of this source is insignificant (see Section 2.3.9), we do not quantitatively model its impact on the prevalence of BSE in the U.S. cattle population or its contribution to contamination of the U.S. food supply.

3.4 Alternative Scenarios Evaluated Using the Simulation Model

The alternative scenarios evaluated using the simulation model fall into three categories. First, we evaluate the plausibility of the model's output by comparing the predicted number of clinical BSE cases to the observed number of clinical BSE cases between 1985 and 2000 in Switzerland (Section 3.4.1). Second, we evaluate the potential for two sources of infectivity (spontaneous disease and cattle imported from the UK during the 1980s) to have introduced BSE into the U.S. prior to the implementation of regulations meant to limit its spread (Sections 3.4.2 and 3.4.3). Finally, we evaluated the extent to which additional risk management actions (implementation of a UK-style specified risk material (SRM) ban, or a ban on the rendering of cattle that die on the farm) reduce the potential spread of BSE among cattle and potential human exposure (Sections 3.4.4 and 3.4.5). Section 4 in Appendix 2 to this report details the parameter file changes made for each of these scenarios.

3.4.1 Switzerland

Because there has never been a controlled experiment to quantify the impact of introducing BSE into a country, a true validation of the simulation model described in this report is not possible. Instead, this section describes an evaluation of the model's plausibility that involves modeling the small BSE outbreak observed in Switzerland following the introduction of BSE infectivity from the UK. Working with experts in Switzerland, we identified appropriate parameter values in order to characterize the herd population dynamics, conditions, practices, and procedures in that country. The Switzerland scenario reflects changing conditions over time. In addition to specifying conditions at the beginning of the simulation (1986), the scenario also reflects changes to these conditions in 1990, 1993, 1996, 1998, and 2001.

This scenario, referred to as "Swiss Best Guess", reflects our best estimate of conditions in Switzerland during the period simulated. After describing this scenario, we outline two modifications ("Swiss Alternative 1" and "Swiss Alternative 2") that were developed after comparing the results of Swiss Scenario to empirical data. Swiss Alternative 1 and Alternative 2 were developed to see whether modest changes to our initial assumptions (modifications that are well within the range of plausibility given our underlying uncertainty) could yield results that are more consistent with these empirical findings.

Swiss Best Guess

1986: The Switzerland scenario begins in 1986, the year we assume that 67 newly infected Swiss female dairy cattle were incubating BSE (Doherr 1999). Thirty of these cattle are assumed to be 25 months of age and the remaining 37 are assumed to be 26 months of age.

At the same time, the Switzerland scenario assumes that feed containing 4,000 cattle oral ID_{50}s was imported. This assumption is based on information that three tons of MBM were imported from the UK between 1985 and 1989. We assume that during that period, MBM from Britain was contaminated with BSE. In particular, we assume that the three tons of MBM imported from Britain represented rendered protein from three cattle, each of which harbored between 800 and 2,000 cattle oral ID_{50}s. We assume that the three tons of MBM were used to supplement feed at a concentration of 5% and was therefore distributed as part of a total of 60 tons of feed. Assuming that cattle consume 30 pounds of feed a day (3% of their weight) and that farms purchase feed in lots sufficient to last them 30 days, the 60 tons (120,000 pounds) of feed would be divided among 133 cattle (*i.e.*, 120,000 pounds ÷ (30 pounds/cow-day × 30 days)).

Differences between the base case and the Switzerland scenario in 1986 include the following. First, the misfeeding rate is assumed to be 15%, considerably higher than the 1.6% misfeeding rate in the base case. The assumption of a substantially higher misfeeding rate is based on the observation that a substantial proportion of the farms in Switzerland raise both livestock that can consume prohibited feed and livestock that are restricted to non-prohibited feed. For example, farm census data suggest that nearly 67% of the poultry in Switzerland are raised on farms that also raise cattle (Heim 2001). For hogs, the corresponding proportion is 59% (Heim 2001).

Second, the Switzerland scenario assumes that most rendering systems in use in 1986 in Switzerland used batch processing technology, which normally reduces infectivity by a factor of 1,000 (*i.e.*, 3 logs). However, because use in Switzerland typically did not conform to the 133°C/20 minutes/3 bars of pressure minimum treatment standard, we assume that the majority of rendering facilities achieved only 2 logs of infectivity inactivation.

Finally, the Switzerland scenario reflects the absence of a feed ban in 1986.

1990: In December, 1990, Switzerland enacted a feed ban and a ban on the rendering or use as human food of SRM, including brain, spinal cord, dorsal root ganglia, gut, lung, eyes, and AMR meat[5]. The structure of the MBM and feed production industries made failures of the ban on the use of SRM in animal feed more likely. In particular, a substantial portion of the prohibited feed was produced by mixed feed producers. We assume that these producers mislabeled or failed to properly label 10% of their prohibited feed and that contamination occurred during production of 20% of the prohibited feed. We also note that increased efforts to keep specified risk materials (SRM) out of the human supply may have increased pressure to divert the flow of this material into MBM and ultimately into animal feed.

1993: By 1993, rendering practices improved. We assume that at that time, all renderers complied with the 133^{o}C/20 minutes/3 bars of pressure standard, and hence that all rendering achieved a 3.1 logs of infectivity reduction (a factor of approximately 1,260).

1996: Changes in farming practices also helped reduce the spread of BSE infectivity. These changes included reduced misfeeding of prohibited rations to cattle (we assume this rate was 0.1%) and eliminating the rendering of cattle that had died on the farm.

1998: In 1998, slaughter facility practices further improved with an increased effort to remove spinal cords after splitting. We assume the spinal cord was removed 99.9% of the time.

2001: Finally, in January, 2001, Switzerland outlawed the practice of feeding MBM to <u>any</u> farm animal[6]. This move essentially eliminated the possibility of misfeeding animals. In addition, Switzerland prohibited the feeding of blood meal to cattle.

<u>Swiss Alternative 1</u>

This scenario is the same as the Swiss Best Guess scenario except that we divided the assumed import of 4,000 cattle oral ID_{50}s equally over three months at the beginning of the

[5] The November, 2001 version of this report assumed that this change occurred in January, 1990, rather than in December of that year. We revised this assumption in response to information from the Swiss Federal Veterinary Service Swiss Federal Veterinary Service (2002). Memo (March 18) to the Harvard Center for Risk Analysis: "Comments on the Harvard study: Evaluation of the potential for Bovine Spongiform Encephalopathy in the United States". .

[6] The November, 2001 version of this report assumed that this change occurred in January, 1999. We revised this assumption in response to information from the Swiss Federal Veterinary Service Ibid..

simulation period (1,333 ID_{50}s per month) rather than assuming that it was all imported in the same month. Doing so substantially increases the number of initial infections that occur at the beginning of the simulation because the original simulation assumed that these 4,000 ID_{50}s were imported in a single month and divided among 133 cattle. Because the size of the exposed group was relatively small for that quantity of infectivity, virtually all the animals received more than 2.0 susceptibility-adjusted ID_{50}s, hence "wasting" infectivity. By dividing the delivery over three months, a total of 399 animals were exposed, hence resulting in a greater number of initial infections.

Swiss Alternative 2

This scenario is the same as the Swiss Best Guess Scenario except that a total of 8,000 ID_{50}s were introduced into cattle feed in 1986 (rather than 4,000), with the import of this infectivity uniformly distributed over a period of 6 months (1,333 ID_{50}s per month).

3.4.2 Spontaneous Disease as a Potential Source of Infectivity in the U.S.

This scenario is the same as the spontaneous disease scenario described in Section 3.3.1 except that it also assumes the absence of the 1997 feed ban. We assume that prior to the adoption of the 1997 feed ban, 65% of the MBM produced by renderers that processed cattle went to animal feed manufacturers, while the remaining 35% was either exported or otherwise allocated to some other use that posed no risk of exposing cattle to BSE infectivity. We further assume that 98% of the feed produced by feed manufacturers was sent to farms and that only 2% was allocated to uses that posed no exposure risk to cattle.

3.4.3 Cattle Imported into the U.S. from the UK During the 1980s

This scenario evaluates the potential consequences of U.S. imports of cattle from the UK during the 1980s prior to the imposition of an import ban in 1989. Of particular concern has been the import 334 cattle from the UK and 162 cattle from the Republic of Ireland during that period because those animals may have been infected with BSE. The vast majority of the cattle imported from Ireland were regarded as posing a negligible risk because they were imported before 1985 and hence before the prevalence of BSE rose sharply in the UK (Section 3.1.1 in (European Union Scientific Steering Committee 2000d)). Of the animals imported from UK, USDA has determined that 161 were disposed of in a manner that eliminates the possibility that

they could have either contaminated the human food supply or lead to the exposure of additional animals in the U.S. to BSE. However, USDA has not been able to conclusively determine that the other 173 animals posed no risk of contaminating either human food or animal feed. This scenario characterizes the potential impact these cattle may have had on the presence of BSE in the U.S.

For each of the 173 animals that may have posed an exposure risk, USDA has determined from Department records and from interviews year of birth, animal type (beef or dairy), gender, age when exported to the U.S., and age when last seen. Using this information, we have computed the probability that the animal was infected and the distribution of values for the animal's total infectivity load. Probabilistically summing these distributions over all 173 cattle yielded a distribution of ID_{50}s imported into the U.S. For this scenario, we assume that all infectivity was imported in 1980. Appendix 5 describes our methodology for developing the imported infectivity distribution.

To determine the impact of these imports, we simulated the introduction of various amounts of infectivity in cattle feed into the U.S. Amounts simulated were 0.1, 1.0, 5.0, 10.0, and 50.0 cattle oral ID_{50}s. The simulation started in the year 1980 and ran through the year 2010. The following assumptions were made for each time period over that 30-year duration.

1980: We assume that at the beginning of the simulation, there was no feed ban in place. In addition, we assume that for cattle between the ages of 12 and 23 months, mis-splits occurred with 5% probability, AMR was used 20% of the time, and spinal cords were removed with 50% probability (regardless of AMR usage). The same assumptions apply to animals 24 months of age and older, except for the mis-split probability, which is assumed to have been 8%. The fraction of spinal cord and DRG that contaminate AMR meat also differs somewhat from the baseline assumptions (see Appendix 2 for details). Finally, we assume that air-injected pneumatic stunning was used for 15% of all animals.

1993: We assume that in 1993, the proportion of animals processed in plants using AMR increased from 20% to 40%.

1997: The simulation reflects implementation of the feed ban in 1997. However, we assume that at this time, the mislabeling rate for prohibited and mixed renderers was 10% (instead

of the base case value of 5%). We also assume that the contamination rate for mixed renderers was 28% (instead of the base case value of 14%). For prohibited and mixed feed producers, we assume that the mislabeling rate was 10% (instead of the base case value of 5%). The probability of contamination for mixed feed producers is assumed to have been 32% (instead of the base case value of 16%).

1999: We assume conditions returned to those characterized by the base case assumptions.

3.4.4 Risk Management: Specified Risk Materials (SRM) Ban

The SRM ban eliminates the potential for the following tissues to contaminate either human food or rendered material that might be used in feed: brain, spinal cord, gut, eyes, and AMR meat products. The SRM ban also eliminates the practice of rendering animals that die on the farm.

3.4.5 Risk Management: A Ban on Rendering Animals that Die on the Farm

Animals that die on the farm are not rendered. We assume that any infectivity in these animals will not contaminate either human food or rendered material that may be used as animal feed.

4 Results

This section highlights key results of the analyses in this report. Complete results can be found in Appendices 3A and 3B. Appendix 3C describes how we have summarized the results generated by the simulation using tables and figures.

Section 4.1 discusses the modeled impact of importing ten BSE-infected animals into the U.S. under present-day conditions (*i.e.*, the base case as described in Section 3.1). The model predicts that such an introduction would be unlikely to result in more than a handful of new cases of BSE, that little infectivity would be likely to reach the U.S. human food supply, and that BSE would likely be cleared from U.S. in less than 20 years.

Section 4.2 describes the results of the sensitivity analyses outlined in Section 3.2. In particular, we describe how altering these assumptions influenced the predicted number of new BSE cases and the amount of infectivity potentially available for human consumption following introduction of ten infected animals. The sensitivity analysis results indicate that the predicted number of additional cattle infected is particularly sensitive to the assumed proportion of prohibited MBM that is mislabeled and the assumed proportion of properly labeled prohibited feed that is incorrectly fed to cattle. Predicted human exposure is likewise sensitive to these parameters. It is also sensitive to the assumed number of ID_{50}s in the carcass of an animal with full blown BSE, and to a lesser extent to several parameters related to the slaughter process.

The results indicate that both the spread of BSE and potential human exposure are proportional to the number of infected cattle introduced into the U.S. We also investigate the impact of importing contaminated feed.

Section 4.3 describes the predicted impact of different sources of infectivity and evaluates both their plausibility and potential for BSE infectivity to spread to cattle or to be available for potential human exposure. The simulation model predicts that under current conditions (i.e., base case assumptions) cross species transmission of scrapie or spontaneous BSE, if they can occur, would produce one to two new cases of BSE per year in the U.S. and little infectivity to humans. Simulations investigating scenarios in which different numbers of infected cattle are imported into the U.S. indicate that both the spread of the disease among cattle and potential human exposure are roughly proportional to the number of infected animals imported.

In all cases tested (up to 500 infected animals imported), the prevalence of BSE decreases over time and tends to be eventually eliminated from the U.S.

Finally, Section 4.4 describes the model's predictions for the scenarios outlined in Section 3.4. The predictions made by the model for the Switzerland scenario are sufficiently similar to those observed to lend the model credibility. Our analysis of potential imports of BSE-infected animals from the UK into the U.S. during the 1980s shows that it is unlikely although not impossible that these imports introduced BSE into the U.S. cattle population. Finally, the simulation's predictions suggest that two risk management measures (a specified risk material ban or a ban on the rendering of cattle that die on the farm) would each further improve defenses against the spread of BSE in this country.

Section 4.5 concludes our report with a summary of the main findings and the implications of BSE for both animal and public health in the U.S.

Before proceeding, we note that many of the simulation results are "right skewed," meaning that the average value often exceeds the median (50^{th}) percentile and can sometimes even exceed the 95^{th} percentile. A right-skewed distribution arises when the lower end of the distribution is bounded (in our case, all of the quantities must be non-negative), and rare events can cause very large outcome values. For example, the probability that the brain of a BSE-infected animal will be selected for potential human consumption is very low because there are few sick animals and few brains harvested for human consumption. However, if this event does occur, it makes a substantial quantity of infectivity available for potential human consumption. If this event occurs only five times in 5,000 simulation runs, the arithmetic mean for the number of cattle oral ID_{50}s available for human consumption from brain would exceed this outcome's value for 4995 of the 5,000 runs (*i.e.*, zero). For this reason, we report key percentile values for each outcome, in addition to the arithmetic mean. Appendix 3C further describes how we have reported the simulation results. The results discussion focuses on mean and median values to characterize the central tendency for each quantity, and the 95^{th} percentile to characterize a quantity's extreme (although not worst possible) case value.

4.1 Base Case

The assumptions that define the base case correspond to contemporary conditions in the U.S., including all risk management actions taken by government and industry. Appendix 1, Section 2 details the corresponding parameter values. Because BSE has not been found in the U.S., the base case is evaluated by assuming the import of ten BSE-infected animals. Such an introduction is considered unlikely because of the ban on importing ruminants from countries known to have BSE. However, this approach allows characterization of the way in which infectivity could spread to animals or humans should the disease be introduced.

The introduction of ten infected animals demonstrates the robustness of U.S. regulations and practices against the establishment of BSE (full results can be found in Section 1 of Appendices 3A and 3B). On average, there are fewer than five new cases of BSE, with a 75% chance that there will be no more than one new case, and at least a 50% chance that there will be no new cases at all. The extreme case (the 95th percentile of the distribution) predicts 16 new cases. The simulation predicts an average of 39 cattle oral ID_{50}s potentially available for human consumption during the 20-year period following the import of the infected animals, with a 95th percentile value of 180 cattle oral ID_{50}s. In all cases, the disease is quickly eliminated from the U.S., with virtually no chance that there are any infected animals 20 years following the import of infected animals.

Potential human exposure routes include consumption of brain (24% of the total on average), contaminated AMR product (51%), beef on bone (12%), intestine (2 %), and spinal cord (10%). Even these estimates are likely to overstate true human exposure because they represent the amount of infectivity *presented* for human consumption but do not take into account waste or actual consumption rates. For example, the reported quantity for potential exposure of ID_{50}s in beef on bone potential reflects the presence of spinal cord and dorsal root ganglia in a fraction of cuts like T-bone steaks. The spinal cord may never be consumed but is still available for potential human exposure. Likewise, not all bovine brain removed for human consumption is actually eaten by humans. Some is not purchased at the retail level and some is not consumed even when purchased. These issues are also relevant to the other tissue categories. For these reasons, our estimates of potential human exposure are likely to overestimate true exposure to infected BSE tissues.

To further characterize the resilience of the U.S. agriculture system, we simulated the impact of introducing 1, 5, 20, 50, 100, 200 or 500 infected cattle (see Section 4.3.3).

4.2 Sensitivity Analyses

This section describes how the use of worst case assumptions in the scenario hypothesizing the introduction of ten infected cattle into the U.S. influences the findings detailed in Section 4.1.

As described below, we find that with three exceptions, the model continues to predict with a high level of certainty that the U.S. agricultural system remains robust against the spread of disease unless worst case values are assigned to multiple parameters simultaneously. In particular, the model's predictions change most dramatically if parameters in the feed production, MBM production, and feed administration practices parameter group are simultaneously assigned worst case values. Because the worst case values are unlikely to be correct for multiple parameters simultaneously, the sensitivity analysis suggests that the findings from Section 4.1 are reasonable. Nonetheless, it would be helpful to develop better information for those parameters that do contribute most substantially to the uncertainty of our findings.

Appendix 3A, Section 2 summarizes the results for each scenario (one table per set of assumptions evaluated). Appendix 3D summarizes the results for each quantity across all scenarios. The results in Section 3D clearly illustrate our finding that most alternative sets of assumption have virtually no impact on the simulation results. Moreover, simultaneously assigning worst case values to both the cattle demographic assumptions and the MBM production, feed production, and feeding practice assumptions has a far greater impact than any other alternative evaluated.

4.2.1 Number of Additional Infected Cattle

As noted in the introduction of Section 3.2, using the base case assumptions results in an R_0 value that is virtually certain to be less than unity, indicating that the prevalence of BSE would decrease over time after being introduced into the U.S. Figure 4-1 illustrates how each alternative worst case assumption individually influences the predicted number of additional new cases of BSE over a 20-year period after the introduction of ten infected animals.

Figure 4-1 can be interpreted by considering an approximate correspondence between the number of additional infected cattle and the value of R_0. Roughly speaking, if R_0 is unity (*i.e.*, each infected animal infects one additional animal), we would expect the number of additional infected animals to be the product of the number of infection cycles and the number of initial animals infected. The infection cycle is the duration between the infection of an animal and that animal's death, at which point the cycle can initiate the next round of infections. The longest this period can be is the length of the incubation period plus the amount of time the animal remains clinical before dying. The average incubation duration is 52 months, while the average time the animal remains clinical is four months. Hence, the average incubation cycle is 56 months long. As a result, there are approximately four full infection cycles per 20 year period, indicating that if R_0 is unity, there should be approximately 40 additional infected animals following the introduction of ten initial infected animals.

We note that this estimate is likely to be conservative because the true infection cycle duration is likely to be less than the average incubation period. For example, in the base case, more than half the infected animals died at slaughter (8.3), rather than on the farm (6.0). The 8.3 animals that died at slaughter produced 1,600 ID_{50}s, far less than would be expected if the animals had survived through the entire incubation period. Hence, an R_0 equal to unity should probably result in more than 40 additional infected animals. Nonetheless, we will use the value of 40 additional animals for the purpose of evaluating the sensitivity analysis findings.

The results illustrated in Figure 4-1 indicate that with the exception of three parameters (3.2..3.1 – Render reduction factor, 3.2.3.5 – Render mislabeling, and 3.2.3.6 – Misfeeding), use of worst case assumptions in place of base case assumptions produces R_0 values that remain below unity with at least 95% probability. Even for these last three parameters, use of worst case values results in R_0 values exceeding unity with less than 25% probability. For example, for the worst case assumptions for misfeeding, the number of additional infected cattle has a 50^{th} percentile value of 1 ($R_0 < 1$), and a 75^{th} percentile value of 16 ($R_0 < 1$). Only the 95^{th} percentile, which is 420, implies an R_0 value exceeding unity. (The mean value is 64). The results also show that with the exception of the render reduction factor paramter, the render mislabeling parameter, and the misfeeding parameter, none of the worst case assumptions substantially change the results distribution, when compared to the base case (first distribution on left side of Figure 4-1).

Figure 4-2 illustrates the impact of assigning worst case values to multiple parameters simultaneously. In this figure, worst case values were assigned simultaneously to all demographic parameters (Section 3.2.1), all slaughter process parameters (Section 3.2.2), and all MBM production, feed production, and feed administration parameters (Section 3.2.3). Assigning worst case values to all demographic parameters has a modest impact on the number of additional infected cattle. The 75^{th} percentile value is 1 (R_0 <1). At the 95^{th} percentile, the number of additional infected cattle (48) slightly exceeds the cutoff we have estimated as corresponding to an R_0 of 1. Setting all slaughter process parameters to their worst case value has a similar modest impact on the number of additional infected cattle. Again, only the 95^{th} percentile (43 additional infected cattle) corresponds to an R_0 value exceeding 1. Because the feed and MBM parameters include the three parameters that had the greatest univariate impact on the number of additional infected cattle (see Figure 4-1), it is not surprising that assigning worst case values to all the parameters in this set has a substantially greater impact on the number of additional infected cattle. Assigning worst case values to all of these parameters simultaneously results in an R_0 value exceeding unity at the 75^{th} percentile.

The three rightmost box and whisker plots in Figure 4-2 illustrate the impact of assigning worst case values to two groups of parameters simultaneously. Assigning worst case values to the demographic parameters and to the slaughter process parameters simultaneously (Sections 3.2.1 and 3.2.2) has only a modest impact on the predicted number of infected cattle. Simultaneously assigning worst case values to the slaughter process and MBM production, feed production, and feed practice parameters (Sections 3.2.2 and 3.2.3) has a somewhat more pronounced impact. The largest impact results when worst case values are simultaneously assigned to all the demographic parameters and to the MBM production, feed production, and feed practice parameters (Section 3.2.1 and 3.2.3). The predicted BSE spread that results is so large that the run time required to simulate this scenario made it impractical to generate 5,000 iterations. Instead, the results reflect a total of 780 iterations. As the detailed results indicate (see Section 2.5.5 in Appendix 3A), with these assumptions the spread of BSE is consistent with an R_0 value that exceeds unity with between 25% and 50% probability. Moreover, the degree to which R_0 can exceed unity in these cases is substantial.

We did not simulate the scenario in which all parameters are simultaneously assigned their worst-case values for three reasons. First, the results described in the preceding paramgraph

indicate that assigning worst case values to two of the three sets of parameters (demographic assumptions and MBM production, feed production, and feed practice parameters) is sufficient to change the predicted behavior of the agricultural system. Second, the extended run time (250 hours on a 3 GHz Windows-compatible PC) needed to generate 780 iterations for this scenario makes testing an even more extreme scenario appear to impractical. Finally, the probability that the worst case values are valid for all parameters seems to be remote.

4.2.2 Infectivity in Food Available for Human Consumption

Figures 4-3 and 4-4 illustrate the results for the univariate and multivariate sensitivity analyses conducted for the estimated number of ID_{50}s in food available for human consumption. Figure 4-3 shows that, as with the number of new infected cattle (Section 4.2.1), use of a worst case assumption for any individual parameter has in most cases a limited impact on potential human exposure to BSE-contaminated food. The only exceptions appear to be two of the influential parameters identified in Section 4.1 (3.2.3.5 – Render mislabeling, and 3.2.3.6 – Misfeeding) and the assumed number of ID_{50}s in the carcass of a full-blown BSE case (3.2.2.1a – ID_{50}s in carcass). In any case, total human exposure over the 20-year period of the simulation remains limited no matter which parameter is assigned its worst case value. Even when the most influential parameter (3.2.3.6 – misfeeding) is assigned its worst case value, the 95^{th} percentile exposure is 1,000 ID_{50}s over 20 years. Lower percentile values were substantially less, with a 75^{th} percentile of 110 ID_{50}s and a median of 21 ID_{50}s.

Figure 4-4 illustrates the impact of assigning worst case values to groups of parameters simultaneously. The results indicate that the demographic parameters (3.2.1) have a limited impact on potential human exposure to BSE-contaminated food, but that collectively, both the slaughter process parameters (3.2.2) and the feed and MBM parameters (3.2.3) have a more substantial impact. Interestingly, although the combination of the slaughter process group parameters and feed and MBM parameters (3.2.2 and 3.2.3) increase the 5^{th}, 25^{th}, 50^{th}, and 75^{th} percentiles to the greatest extent (compared to the base case results), the combination of the demographic parameters and feed and MBM parameters (3.2.1 and 3.2.3) increase the 95^{th} percentile, and consequently the arithmetic mean, to the greatest extent.

4.3 Alternative Sources of Infectivity

We evaluate three potential sources of BSE in the U.S. Section 4.3.1 considers the impact of assuming BSE can develop spontaneously in cattle with an incidence rate that mirrors

the age-specific incidence of CJD in humans. Section 4.3.2 considers the import of various numbers of infected cattle (1, 5, 20, 50, 200, and 500) and the import of contaminated feed (10,000 ID_{50}s). Finally, Section 4.3.3 considers the impact of assuming that scrapie can be transmitted from sheep to cattle. In all of these cases we assume the conditions specified in the base case hold.

4.3.1 Spontaneous BSE

For this scenario, the model predicts an average of 27 infected animals over a 20-year period (95^{th} percentile value of 38). It is predicted that only 2.7 animals, on average, would reach the clinical stage of the disease (95^{th} percentile of 6). Virtually all animals that become infected develop the disease spontaneously, although maternal transmission and transmission caused by contaminated protein both make a small contribution. The simulation predicts that a mean of 73 cattle oral ID_{50}s would be potentially available for human consumption (95^{th} percentile value of 220).

These results suggest that if this hypothesis is true, the disease is essentially endemic, with one-to-two cases occurring each year. Current agricultural practices and regulations (the feed ban) effectively check the spread of disease to other cattle but the disease cannot be eliminated because of its sporadic occurrence. The very low number of animals developing clinical signs would make detection using any method of surveillance very difficult.

4.3.2 Imports

Figures 4-5, 4-6, and 4-7 respectively illustrate the relationship between the number of infected cattle imported and the number of new cases (*i.e.*, the number of cases in addition to the imported animals) during the 20 year period following the arrival in the U.S. of these imports, potential human exposure to BSE during this period, and the probability that BSE will be present in the U.S. at the end of the 20-year period. In Figures 4-5 and 4-6, the medians are connected by a solid line. The results indicate that all three outcomes increase linearly as a function of the number of infected cattle introduced. Most importantly, Figure 4-7 shows that even after the introduction of 500 cattle, the probability that BSE is still present in the U.S. after 20 years has dropped to approximately 10%. This finding suggests that the prevalence of BSE decreases over time regardless of how large the introduction is. That is, the value of R_0 remains less than one.

We note also that following the introduction of contaminated feed containing 10,000 ID_{50}s, the median simulation predictions are: a total of 1,600 cattle infected over 20 years, potential human exposure to approximately 4,300 cattle oral ID_{50}s, and that after 20 years, an 18% chance that BSE still remains in the U.S. By comparing these results to the median predictions in Figures 4-5 and 4-6, and the probability predictions in Figure 4-7, we can characterize the impact of importing contaminated feed in terms of the number of infected cattle that would have the same impact. Assuming the linear relationships in these figures hold at higher levels, the import of 10,000 ID_{50}s has the same impact on the spread of BSE (newly infected cattle) as importing 3,600 infected cattle. It has the same impact on human exposure as importing 1,100 infected cattle. Finally, it has the same impact on the persistence of the disease (*i.e.*, probability that it is present in the U.S. after 20 years) as the import of 820 infected cattle.

4.3.3 Scrapie

This simulation evaluates the impact of assuming that scrapie contributes one cattle oral ID_{50} to feed consumed by cattle each month. The simulation predicts that this contamination results in an average of 38 infected cattle over a period of 20 years (95^{th} percentile estimate of 64). The simulation also predicts that an average of about six animals would develop clinical signs during that period (95^{th} percentile of 12). Current surveillance would be unlikely to detect this number of clinical cases. On average, the simulation predicts that approximately 100 cattle oral ID_{50}s would be available for potential human exposure during the 20 y ear period (95^{th} percentile estimate of 290).

Because scrapie is assumed to contaminate cattle feed continually, the disease would essentially be endemic. Note that the simulation predicts that most new cases of BSE would arise directly from exposure to scrapie infectivity, although a small number of cases would result from exposure to contaminated ruminant protein that slips through the feed ban. Maternal transmission also makes a small contribution to the total.

We expect that the predictions made here are likely to overstate the true contribution of scrapie to BSE, as explained in Section 3.3.3. In brief, it is likely that the true species barrier is greater than the value of 1,000 used (efforts to transmit North American scrapie orally to cattle have produced negative results in all instances), and the prevalence of scrapie in the U.S. is

probably less than the UK prevalence rates used in the calculation. Section 3.3 of Appendices 3A and 3B detail the simulation results.

4.4 Alternative Scenarios

This section details the results of several simulations designed to investigate further factors influencing spread of BSE infectivity. The first scenario described models the small BSE outbreak in Switzerland to evaluate the plausibility of our model (section 4.4.1). Next we examine the spontaneous hypothesis by looking at how spontaneous disease might have spread in the years before the FDA feed ban was adopted (section 4.4.2). Section 4.4.3 examines how the import of cattle from the UK during the 1980s may have affected the U.S. The last two sections evaluate specific risk management strategies, including a specified risk material (SRM) ban identical to that imposed in the UK (Section 4.4.4), and a prohibition on the rendering of animals that die on the farm (Section 4.4.5).

4.4.1 Switzerland

As discussed in Section 3.4.1, our model is not amenable to formal validation because there have been no controlled experiments in which the consequences of BSE introduction into a country have been monitored and measured. However, as a test of the model's plausibility, we modeled the small BSE outbreak reported in Switzerland following the introduction of BSE infectivity from the UK. Our simulation took into account risk management actions taken by the Swiss during the ensuing period (*e.g.,* the introduction of a feed ban regulation).

The model predicts both the total number of infected animals in Switzerland and the incremental number that develop clinical signs of disease. Only animals with clinical signs could be detected using the standard surveillance methods available early in the outbreak (although current surveillance practices can detect disease in animals several months before development of clinical signs). We therefore compare the monthly clinical case incidence predicted by the model to the empirical clinical case incidence estimates reported by Doherr *et al.* (1999). As illustrated in Figure 4-8, the modeled incidence rate increases above zero around two years before the empirical rate, peaks at about one-half the empirical rate, and declines to zero at around the same time the empirical rate declines to zero. The modeled cumulative incidence is approximately 60% the empirical cumulative incidence. If empirical counts reflect underreporting, the actual incidence of clinical cases may exceed the modeled incidence by an even greater degree than the

approximate factor of two suggested by Figure 4-8. Doherr *et al.* raise the possibility of underreporting by as much as 75%. However, Doherr *et al.* suggest that these substantial underreporting rates most likely apply to "*cases late in incubation or with early clinical signs*" (p. 159). It is therefore plausible that the overall underreporting rate for clinical cases would be much lower.

Even without an adjustment for potential underreporting, the modeled estimates described above understate the empirically reported case incidence rate. However, as described in Section 3.4.1, these modeled values reflect an initial best-guess set of assumptions with no adjustments made to try to match the empirical counts. Our results indicate that only modest changes to the assumptions (Swiss Alternative 1 and Swiss Alternative 2) are needed to achieve such a congruence. Given the level of uncertainty associated with the scenario-specific assumptions, the results in Figure 4-9 indicate that the Alternative 1 assumptions produce results that come reasonably close to matching the empirical counts. The Alternative 2 assumptions produce results that come reasonably close to matching twice the empirical counts. Complete simulation results appear in Section 4.1 of Appendices 3A and 3B.

Our model's modest underprediction of clinical cases could be due to incorrect specification of the number of infected animals imported or the amount of contaminated feed introduced, among other factors. At the same time, the similarity of our predictions and the observations from Switzerland provide some confidence that the model's structure and approach are reasonable. It is important to note that this is not a true validation and, in fact, the model's predictions could be close to reported observations for the "wrong reasons." However, given the absence of data suitable for validating the model, the results of the Switzerland scenario are encouraging.

4.4.2 Spontaneous With no Feed Ban

To further investigate the spontaneous hypothesis, we modeled a scenario in which spontaneous disease occurs using the rates described in Section 3.3.1, but no feed ban is present to mitigate the recycling of infectivity in ruminant feed. The scenario, described in Section 3.4.2 was run for 20 years.

The absence of a feed ban allows BSE infectivity to rapidly spread throughout the cattle population. The mean projection for this scenario suggests 42,000 animals infected over the 20 year period (95th percentile of 190,000). The average number of clinical animals predicted is 1,500 (95th percentile of 6,600).

It should be noted that the simulation often predicts that the BSE prevalence rapidly increases towards the end of the twenty year period (see Section 4.2 in Appendices 3A and 3B for complete results). This tendency suggests that if a longer time period were simulated, the model would predict a much greater burden of disease. Hence, while some simulation runs predict prevalence rates that are low enough to be compatible with the fact that BSE has not been detected in the U.S., the results suggests that even in these cases, the prevalence would climb much higher if a longer period were simulated. That is, in the absence of a feed ban, the prevalence would most likely reach a detectable level in any case in just over 20 years. The fact that BSE was not detected in the U.S. prior to the implementation of the feed ban therefore suggests that either spontaneous disease either does not occur, or that its incidence is less than we have assumed. Alternately, the imposition of the feed ban may have stopped an epidemic before it could reach detectable levels. In that case, the base case results suggest that the feed ban will eliminate the disease shortly.

4.4.3 Cattle Imported from the UK in the 1980s

This scenario investigates the likelihood that BSE infectivity could have been introduced into the U.S. by the import of 173 cattle from the UK during the 1980s that may have contaminated either human food or animal feed (see Section 3.4.3). We also determine the amount of infectivity that may have been introduced. Using these findings, we characterize the likelihood that BSE could have been introduced into the U.S. and remained undetected.

As discussed in Section 3.4.3, some of the cattle imported into the U.S. from the UK between 1980 and 1989 may have been infected with BSE without showing clinical signs of the disease. As a result, diseased animals may have contaminated animal feed in this country. Figure 4-10 illustrates the cumulative distribution for the amount of infectivity (cattle oral ID_{50}s) that may have been in feed consumed by cattle in the U.S. (see methodology in Section 3.4.3 and in Appendix 5). The distribution indicates it is likely (probability of 82%) that U.S. cattle were exposed to no infectivity from cattle imported from the UK. The probability that cattle were

exposed to no more than 0.1 ID_{50}s is 84%, the probability that they were exposed to no more than one ID_{50} is 86%, the probability that they were exposed to no more than five ID_{50}s is 91%, the probability that they were exposed to no more than ten ID_{50}s is 93%, and the probability that they were exposed to no more than 50 ID_{50}s is 96%.

To characterize the impact of introducing infectivity into the U.S. during the 1980s, we have simulated the introduction of 0.1, 1.0, 5.0, 10.0, and 50.0 cattle oral ID_{50}s into cattle feed in 1980, and followed the evolution of the U.S. cattle population through 2010. The results of these simulations (see Section 4.3 in Appendices 3A and 3B) can be used to quantify the likely number of clinical BSE cases that would have occurred and hence to assess the plausibility of these scenarios in light of the fact that BSE has not been detected in the U.S. In particular, introductions that result in too large a number of clinical cases to be compatible with the fact that BSE has not been detected in the U.S. are not plausible.

Note that the distributions for the output quantities are highly skewed, indicating that under most circumstances the infectivity did not spread widely but that occasionally, there was a combination of events leading to significant numbers of infected cattle. For example, when 0.1 cattle oral ID_{50} is introduced into feed, more than 4,750 of the 5,000 simulation runs for this scenario produced no new cases of disease. However, a few runs produced substantial numbers of diseased animals. Hence the mean number of infected animals (over all 5,000 simulations) is 45, and the mean number of animals with clinical signs is ten. Introducing larger quantities of infectivity also yields right-skewed results distributions.

The probability that BSE was introduced into the U.S. depends on two events – the introduction of contaminated material from imported animals into domestic cattle feed (probability of 18%), and the infection of exposed cattle and the subsequent spread of BSE to other animals without the creation of so many cases that it would have been likely to have been discovered by surveillance. Figure 4-11 illustrates for the year 2000 (year 20 of the simulation) the predicted number of cattle with clinical signs following the introduction of 0.1, 1.0, 5.0, 10.0, or 50.0 cattle oral ID_{50}s from the imported UK animals into feed administered to U.S. cattle in 1980. Also plotted is the USDA's estimate of the number of clinical cases surveillance would have detected in the year 2000 with 95% probability based on the methods and level of surveillance at the time (Bridges 2001; U.S. Department of Agriculture 2002). For example, the curve in Figure 4-11 corresponding to the introduction of 10.0 ID_{50}s indicates that there is an 82%

chance that this introduction caused no new BSE cases in the U.S.[7], and that it could have resulted in a maximum of approximately 1,100 clinical cases in the year 2000. However, all values exceeding the detection limit of 470 clinical cases in the year 2000 (*i.e.*, above the horizontal "detection limit" line) are implausible because no BSE has been detected in the U.S. For the introduction of 10.0 ID_{50}s, there is a 6% chance that the number of clinical cases in 2000 would have exceeded this limit (*i.e.*, a 94% chance that this value would have been below the detection limit). Hence, even if cattle in the U.S. did consume 10.0 ID_{50}s in 1980, there is only a 12% chance (94% minus 82%) that it resulted in BSE cases that have not been found. Corresponding probabilities can be computed for the other ID_{50} introductions considered.

Taken together, Figures 4-10 and 4-11 are useful for evaluating the likelihood that BSE cattle imports from the UK during the 1980s introduced BSE into the U.S. but resulted in too few cases for the disease to have been detected. First, there is only an 18% chance that cattle in the U.S. were exposed to any infectivity (see Figure 4-10). Second, if cattle were exposed to infectivity, there is only a limited probability that both 1) any cattle in the U.S. became infected, and 2) the number of clinical cases (in the year 2000) was less than the number that would have been likely to have been detected (see Figure 4-11).

Finally, the Figures in Section 4.3 of Appendix 3B illustrate how the disease spreads and contracts if it is introduced into the U.S. The figures suggest that the number of animals with detectable disease peaks in year 20 of the simulation (calendar year 2000) and declines thereafter. This prediction indicates that even if infectivity has been introduced from UK cattle imported before 1989, the disease rate has peaked and BSE will eventually be eradicated. The decline in the predicted disease prevalence in the U.S. is due primarily to the introduction of the FDA feed ban in 1997.

4.4.4 Specified Risk Material Ban

Many countries with BSE have prohibited the use of certain tissues in either animal feed or human food. These specified risk material (SRM) bans focus on tissues carrying the greatest

[7] Figure 4-9 illustrates the number of clinical cases in the year 2000, not the total number of BSE cases caused by the import of BSE-infected cattle from the UK. However, the scenario simulated assumes that action to mitigate the spread of BSE in the U.S. occurs only after implementation of the feed ban in 1997. Hence, as suggested by the figures in Section 4.3 of Appendix 3B, the number of clinical animals peaks

amount of BSE infectivity. To evaluate how such a ban would influence the spread of BSE in the U.S., we altered the base case scenario as described in section 3.4.4 to mimic the UK SRM ban.

The SRM ban has a dramatic effect on both potential human exposure and the spread of BSE among cattle. Following the introduction of 10 infected cattle, as in the base case, the mean number of new BSE cases is reduced by nearly 90% (from 4.3 to 0.53) and the mean number of cattle oral ID_{50}s potentially available for human exposure decreases by 95% (from 39 to 1.8). Results for this scenario appear in Section 4.4 of Appendices 3A and 3B.

4.4.5 Prohibition on Rendering Animals that Die on the Farm

The results for the base case simulation (section 4.1 and Section 1 in Appendices 3A and 3B) clearly indicate that if BSE is introduced into the U.S., the greatest potential source feed contamination is animals that die prior to being sent to slaughter (animals that die on the farm) and are rendered. The simulations in this report assume that an animal lives for between two and six months following the development of clinical signs. Rendering an animal that has reached the clinical stage of disease introduces the maximum amount of infectivity into rendering and potentially into feed. Hence, a single breach of the feed ban can introduce expose cattle to a substantial amount of BSE infectivity.

The simulation results indicate that banning the rendering of animals that die on the farm would substantially reduce the spread of BSE to other cattle following introduction of ten infected cattle. Compared to the base case, the mean number of new cases decreases by more than 80% (from 4.3 to 0.77). Although this approach targets the spread of BSE to other animals, it also influences potential human exposure to BSE infectivity, decreasing this quantity by more than 20% because it decreases the number of new BSE cases. Complete results appear in Section 4.5 of Appendices 3A and 3B.

4.5 Summary

This report addresses the potential for BSE to become a major animal health problem or substantially contaminate the human food supply in the U.S. The results characterize the

---

around the year 2000. As a result, if there are zero clinical animals in the year 2000, it is almost certain that few if any animals were infected in the U.S.

robustness of regulations and practices in the U.S., and help to identify data or research that would most increase confidence in our predictions. In addition, the results help to characterize the potential impact that various sources of BSE may have had in the U.S. in the past, including cattle imported from the UK in the 1980s. Finally, the simulation can be used to characterize the effectiveness of additional risk management strategies.

We recognize that the identification of a single case of BSE in the U.S. would have important ramifications for public opinion, trade, and other areas. Yet this analysis demonstrates that even if BSE were somehow to arise in the U.S., few additional animals would become infected, little infectivity would be available for potential human exposure, and the disease would be eradicated. In short, the U.S. appears very resistant to a BSE challenge, primarily because of the FDA feed ban, which greatly reduces the chance that an infected animal would infect other animals. However, the effectiveness of the feed ban is somewhat uncertain because compliance rates are not precisely known.

Potential sources of human exposure to BSE infectivity can be divided into two categories: specific high-risk tissues and contamination of low-risk tissues. Although not widely popular in the U.S., both brain and spinal cord are consumed by some members of the population. If BSE were present in the U.S., these tissues would be an obvious source of exposure. Our analysis indicates that the most important means by which low risk tissue can become contaminated is the use of advanced meat recovery (AMR) technology, which can leave spinal cord or dorsal root ganglia (DRG) in the recovered meat. Our analysis further indicates that mis-splitting of the spinal column and the resulting incomplete removal of the spinal cord is largely responsible for contamination of AMR meat. In addition, we assume that even in the absence of mis-splitting, some amount of DRG is extracted whenever vertabrae are processed by AMR. Contamination due to aerosolization of the spinal cord during splitting contributes substantially less contamination even though it occurs every time an infected animal is processed.

Despite the potential for the consumption of high risk-tissues and the contamination of low-risk tissues, our results indicate that only small amounts of infectivity are available for human consumption. The import of one infected animal yields an average of 3.7 cattle oral ID_{50}s for potential human exposure over a 20 year period, while the import of ten infected cattle results in an average of 39 cattle oral ID_{50}s this period. These results can be put into context by comparing them to potential human exposure in the UK where it is estimated almost one million

cattle were infected over a 15 to 20 year period. If the UK population was potentially exposed to only one cattle oral ID_{50} from each of these animals, potential human exposure in the UK would dwarf our projections for the U.S. At this time, just over 100 cases of variant Creutzfeldt-Jakob disease (the human TSE linked to BSE) have been identified in the UK, although projections range from a few hundred to tens of thousands of eventual cases. If cattle oral ID_{50}s available for human consumption is a good indicator of possible disease risk, it is unlikely the UK experience would be duplicated in the U.S.

There are a number of model assumptions that cannot be verified with confidence, some of which substantially influence the conclusions drawn. With regard to estimating the spread of BSE among cattle, the most influential sources of uncertainty are related to compliance with the FDA feed ban. Within this category, the most important source of uncertainty is the misfeeding rate on farms. Misfeeding prohibited feed (containing ruminant protein) to cattle on farms that raise both cattle and either pigs or chickens completely compromises the feed ban. This practice is the focus of efforts to understand how animals born after the implementation of feed bans in Europe have become infected with BSE. Uncertainty with respect to compliance rates can be reduced with field work and data collection. A second source of uncertainty associated with the feed ban is the proportion of feed produced that is mislabeled (*i.e.*, lacks the proper labels identifying it as feed not to be administered to ruminants). Finally, assumptions regarding the prevalence of alternative rendering technologies used (and hence the degree to which rendering may reduce the level of infectivity in tissue processed to produce MBM) also influence the predicted spread of BSE.

Improving estimates of compliance with the feed ban would also improve the precision of our estimates of potential human exposure to BSE-contaminated meat. The assumed number of ID_{50}s per clinical case of BSE also has a notable impact on predicted potential human exposure to BSE.

We have identified three important ways in which BSE could be introduced into the U.S: 1) cross-species transmission from a native TSE like sheep scrapie, 2) spontaneous development of the disease in native animals, or 3) the import of an infected animal or animal product from a country with BSE. The analysis suggests that either cross-species transmission of a TSE (scrapie) or spontaneous disease, if they can occur, would cause only a few cases of BSE each year and would result in relatively little potential human exposure. However, results from our evaluation

of the impact of spontaneous BSE on the U.S. prior to the 1997 FDA feed ban casts doubt on the plausibility of this potential source of BSE. In particular, our results suggest there is a substantial probability that the number of animals with clinical signs would be sufficiently high to be inconsistent with the fact that surveillance has failed to detect BSE in the U.S. At the same time, the simulation results indicate that there is a non-trivial probability that spontaneous BSE would generate an insufficient number of animals to be detected by surveillance.

Although it is not possible to know if an infected animal was imported from the UK in the 1980s, our analysis suggests it is highly unlikely. First, the imported animals whose disposition is not known came from farms where the disease was not found in any animal born during the same year. Second, the beef breeding animals imported had little exposure to potentially infected protein supplements while in the UK. Finally, many of the animals are known to have lived beyond the average incubation period once they arrived in the US. Nonetheless, there is some small probability that at least one of these animals was infected and that infectivity from such an animal contaminated feed consumed by cattle in the U.S. Exposure to infectivity among U.S. cattle could not have been substantial because in the years prior to the 1997 FDA feed ban, such exposure would have eventually resulted in a substantial number of clinical cases, a prediction that is inconsistent with the fact that BSE has not been identified in the U.S. to date. There is therefore a small chance that BSE could have been introduced into the U.S. and remained undetected. Even if BSE was introduced, actions by USDA and FDA have already arrested the spread of the disease and have begun to reduce its prevalence. If BSE is present in the U.S., these actions will ultimately lead to the disease's eradication.

Evaluation of potential risk management actions highlights an additional benefit of this type of analysis. The insights provided by the model demonstrate that interventions very early in the rendering and feed production process can avoid the need for other, more obvious, measures. Specifically, removing most of the infectivity from rendered product can protect human and animal health even if the feed ban is not 100% effective. Both disposing of all specified risk materials and prohibiting the rendering of animals that die prior to being sent to slaughter, *i.e.*,animals that may have died of BSE and hence have high levels of infectivity, reduce potential new cases of BSE by more than 80%. The misfeeding rate, a key parameter identified in our sensitivity analysis, is not important if the infectivity in prohibited MBM is greatly reduced or eliminated. The SRM ban also reduces substantially the amount of infectivity available for potential human exposure. Of course, it must be recognized that even in the absence of these

measures, animal health risks and human exposure are both small, with the import of ten infected cattle leading to an average of fewer than five new cases of BSE and potential human exposure to 39 cattle oral ID_{50}s.

As we strive to learn more about BSE and limit the extent of the disease, the model developed for this analysis has many potential uses. It is flexible and can be changed easily. For example, if appropriate data are available, its parameters can be modified so that other countries or regions can be simulated. Specific scenarios of interest can be evaluated, including risk management actions under consideration. The model can also be used to evaluate hypotheses about sources and factors influencing the BSE's spread. We hope this model will find a place among the useful tools for understanding and controlling BSE.

Glossary

AMR (Advanced Meat Recovery) – FSIS (U.S. Department of Agriculture (FSIS) 2002) states that "*AMR systems remove the attached skeletal muscle and edible tissues from carcasses without breaking or crushing bones. This machinery separates meat by scraping, shaving or pressing the muscle and edible tissue away from the bone. However, unlike traditional mechanical separation, AMR machinery cannot break, grind, crush or pulverize bones to separate muscle tissue. Bones must emerge essentially intact and in natural physical conformation.*"

APHIS (Animal Plant Health Inspection Services) – APHIS is an agency that is part of the U.S. Department of Agriculture.

BSE (Bovine Spongiform Encephalopathy) – BSE is a slowly progressive and fatal prion disease of adult cattle. The disease is characterized for spongy changes in the brain and a long incubation period.

BSE Inquiry - Inquiry established by the UK Prime Minister to investigate the emergence and identification of Bovine Spongiform Encephalopathy (BSE) and variant Creutzfeldt Jakob Disease (vCJD) as well as the government response. The Inquiry was established on March 20, 1996.

Bypass protein – Bypass protein is the feed protein that escapes digestion in the rumen and passes into the lower digestive tract where is digested and absorbed. Bypass proteins are important proteins in the nutrition of dairy animals.

CJD (Creutzfeldt-Jakob Disease) – CJD is a fatal prion disease that has been known for many years to affect human. It can be transmitted as the result of consuming contaminated tissue (as part of canabilistic rituals) or when contaminated tissue is used in surgical procedures.

CNS (Central Nervous System) – The CNS consists of nervous tissue that includes brain and spinal cord.

Codon – A series of 3 successive nucleotides in nucleic acid that specifies a particular amino acid or signal sequence in a protein.

CWD (Chronic Wasting Disease) – CWD is a prion disease that affects white tail deer, mule deer and elk. The disease has been found only in North America.

Distal Ileum – The distal ileum is the lower portion of the small intestine.

Downer Cattle – See "non-ambulatory cattle."

DRG (Dorsal Root Ganglia) – DRG are the nervous tissue that are located within the bones of the vertebral column. DRG contain nerve cells that transfer sensory signals from parts of the body to the spinal cord.

FDA – U.S. Food and Drug Administration

FFI (Fatal Familial Insomnia) – FFI is a rare human familial prion disease.

FSE (Feline Spongiform Encephalopathy) – FSE is a prion disease that affects cats. Exposure to the BSE agent is the most likely explanation for the emergence of the disease.

FSIS (Food Safety and Inspection Service) – FSIS is an agency that is part of the U.S. Department of Agriculture.

Genotype – Genetic constitution of an individual organism. In particular, this term refers to the specific chromosomal alleles that determine specific traits.

GSS (Gerstmann-Sträussler-Scheinker) – GSS is a rare familial prion disease that affects humans.

HCRA – Harvard Center for Risk Analysis.

Heterozygous – This term refers to organisms that have two different alleles of the same gene.

Histopathology – The study of microscopic changes in diseased tissues.

Homozygous – This term refers to individuals that have two identical alleles of the same gene.

Horizontal transmission – Transmission within a population other than by genetic or maternal means.

i.c. (intracerebral) inoculation – Injection into the brain

ID_{50} (Infectious Dose 50) – An ID_{50} is the amount of infectious material (e.g., infective bovine brain) that when consumed results in disease infection with 50% probability. The amount of material that constitutes one ID_{50} depends on the route of exposure (*e.g.*, oral administration or intracerebral inoculation).

Immune response – This response is the action taken by the body to minimize the damage resulting from the presence of a foreign agent in the body.

Immunohistochemistry – Techniques for staining cells or tissues using labeled antibodies against specific proteins.

Incubation period – The period between infection and clinical manifestation of the disease.

Infectivity – Infectivity is a general term referring to the agent that is capable of passing on disease.

i.p. (intraperitonal) inoculation – Injection into the abdominal cavity.

Kuru – Kuru is a rare human prion disease found in the Fore population of Papua, New Guinea.

Mad-Cow Disease – The colloquial name for bovine spongiform encephalopathy.

MAFF (The UK Ministry of Agriculture, Fisheries and Food) – MAFF has been renamed the Department of Environment Food and Rural Affairs (DEFRA).

Maternal Transmission – Transmission from dam to offspring *in utero* (during pregnancy) or during the intermediate post partum period

MBM (Meat-and-bone meal) – MBM is a dried homogenized material produced by rendering animal tissues. MBM is used as a protein source in the production of animal feed.

MRM (Mechanically Recovered Meat) – MRM is defined as "…*residual material, off bones, obtained by machines operating on auger, hydraulic or other pressure principles in such a manner that the cellular structure of the material is broken down sufficiently for it to flow in puree form from the bone*" (BSE Inquiry 2000b).

Non-ambulatory cattle – Non-ambulatory cattle include animals that are unable to rise. This condition is common and usually affects animals around parturition. It can also result from a variety of causes, including neurological disease.

Non Prohibited Feed – Non-prohibited feed does not contain proteins derived from ruminants and/or mink and can therefore be legally administered to ruminants.

Non Prohibited MBM – Non-prohibited meat and bone meal does not contain proteins derived from ruminants and/or mink and hence can legally be used in the preparation of ruminant feed.

OIE (Office International des Epizooties) – OIE determines animal health standards for international trade, advises the veterinary services in member countries and aims to work towards the eradication of the most dangerous animal and zoonotic diseases. As of May, 2003, 164 countries belonged to the OIE.

Pathogenesis – This term refers to the process by which disease develops in an organism.

Pre-clinical – Refers to the disease stage prior to the manifestation of clinical signs or symptoms.

Prion Disease – Prion diseases are a family of fatal brain diseases that occur in a number of mammals including humans. These diseases are also known as Transmissible Spongiform Encephalopathies (TSE's). Prion diseases are caused by the build-up of abnormal proteins in the central nervous system.

Prohibited Feed – Prohibited feed contains ruminant protein or mink protein and therefore cannot be legally used to produce feed for ruminants.

Prohibited MBM – Prohibited meat and bone meal contains ruminant protein or mink protein and therefore cannot be legally used to produce feed for ruminants.

PrP (Prion Proteins) – Prions are proteins that occur naturally in animals and humans. Research suggests that if a prion is folded incorrectly and hence has an abnormal shape, it can induce disease. Moreover, when mis-shaped proteins come into contact with normal proteins, they can "recruit" the normal proteins, causing them to become mis-shaped. Some scientists believe that if this process progesses sufficiently, prions can damage the brain, causing it to become spongy and filled with holes. This phenomenon gives rise to the scientific name for mad cow disease (bovine spongiform encephalopathy).

PrP^{C}, PrP^{Sen} – The normally folded form of PrP.

PrPgene – Gene found in mammals that determines the amino acid sequence for the PrP^{C} protein.

PrP^{Sc}, PrP^{Res} – The abnormally folded disease-specific isoform of PrP.

Rendering – Rendering is processing of offal and discarded parts of animal carcasses to produce two products: meat and bone meal (MBM) and tallow. The rendering process consists of drying, cooking, and separating the solid fraction (protein meals) form the melted liquid fraction (tallow).

Ruminant – Animal that chews the cud (partially digested food) regurgitated from its rumen, and has a stomach with four compartments.

Scrapie – Scrapie is a prion disease that affects sheep and goats.

SEAC (Spongiform Encephalopathy Advisory Committee) – Established in UK to advise the government on matters related to TSEs (prion diseases).

Spinal Cord – The part of the nervous system that runs through the spine or vertebral column.

SRM (Specified Risk Material) – Tissues in in cattle, sheep and goats such as brain tissue and spinal cord, that are most likely to contain the BSE infective agent.

SSC (Scientific Steering Committee) – Established in the European Community to advise its members on matters related to TSE's (prion diseases) and other zoonoses.

Tallow – The fat produced by the rendering process.

TME (Transmissible Mink Encephalopathy) – TME is a prion disease that affects mink. The disease has been found several countries.

TSEs (Transmissible Spongiform Encephalopathies) – See prion disease.

USDA – United States Department of Agriculture

vCJD (variant Creutzfeldt-Jakob Disease) – vCJD is the name of the human prion disease that is thought to be caused by consumption of BSE-contaminated meat.

Vertebral Column – The supporting line of bones that make up the spine and house the spinal cord.

Vertical Transmission – Transmission of disease from parent to the offspring. See also maternal transmission.

Western Blot – A method use for detecting proteins, including diseased PrP. This method can be used to diagnose TSEs.

WHO – World Health Organization.

Zoonosis - Animal diseases that can be transmitted to humans.

References:

Agrimi, U., Ru, G., Cardone, F., Pocchiari, M. and Caramelli, M. (1999). Epidemic of transmissible spongiform encephalopathy in sheep and goats in Italy. *Lancet* **353**(9152): 560-1.

Airtime and Resources, I. (2001). Hypotheses for the origin and spread of BSE. *http://sparc.airtime.co.uk/bse/hypoth.htm#15*.

Alpers, M. (1970). Kuru in New Guinea: its changing pattern and etiologic elucidation. *American Journal of Tropical Medicine & Hygiene* **19**(1): 133-7.

Anderson, R. M. and May, R. M. (1991). *Infectious Diseases of Humans: Dynamics and Control*, Oxford University Press.

Anderson, R. M., Donnelly, C. A., Ferguson, N. M., Woolhouse, M. E., Watt, C. J., Udy, H. J., MaWhinney, S., Dunstan, S. P., Southwood, T. R., Wilesmith, J. W., Ryan, J. B., Hoinville, L. J., Hillerton, J. E., Austin, A. R. and Wells, G. A. (1996). Transmission dynamics and epidemiology of BSE in British cattle. *Nature* **382**(6594): 779-788.

Andreoletti, O., Berthon, P., Marc, D., Sarradin, P., Grosclaude, J., van Keulen, L., Schelcher, F., Elsen, J. and Lantier, F. (2000). Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. *Journal of General Virology.* **81**(12): 3115-26.

Anil, M. H., Love, S., Williams, S., Shand, A., McKinstry, J. L., Helps, C. R., Waterman-Pearson, A., Seghatchian, J. and Harbour, D. A. (1999). Potential contamination of beef carcases with brain tissue at slaughter. *Veterinary Record* **145**(16): 460-462.

anonymous (1996). Surveillance for Creutzfeldt-Jakob disease--United States. *MMWR - Morbidity & Mortality Weekly Report* **45**(31): 665-8.

Belt, P., Muileman, I., Schreuder, B. E. C., Ruijter, J. B., Gilkens, A. L. J. and Smits, M. A. (1995). Identification of five allelic variants of sheep PrP gene and their association with natural scrapie. *Journal of General Virology* **76**(509-517).

Biopharm (1997). Assessment of the Risk of Bovine Spongiform Encephalopathy in Pahrmaceutical Products. *http://www.biopharm-mag.com/resources/pharma0198.htm*.

Bolton, D. C., McKinley, M. P. and Prusiner, S. B. (1982). Identification of a protein that purifies with the scrapie prion. *Science* **218**(4579): 1309-11.

Borras, T. and Gibbs, C. J., Jr. (1986). Molecular hybridization studies with scrapie brain nucleic acids. I. Search for specific DNA sequences. *Archives of Virology* **88**(1-2): 67-78.

Bossers, A., Belt, P., Raymond, G. J., Caughey, B., de Vries, R. and Smits, M. A. (1997). Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms. *Proceedings of the National Academy of Sciences of the United States of America* **94**(10): 4931-6.

Bradley, R. (1999). BSE transmission studies with particular reference to blood. *Developments in Biological Standardization* **99**: 35-40.

Brewer, R. (2000). U.S. Department of Agriculture, Food Safety and Inspection Service. Personal Communication.

Bridges, V. (2001). U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Centers for Epidemiology & Animal Health. Personal Communication.

Brown, P., Rodgers-Johnson, P., Cathala, F., Gibbs, C. J., Jr. and Gajdusek, D. C. (1984). Creutzfeldt-Jakob disease of long duration: clinicopathological characteristics, transmissibility, and differential diagnosis. *Annals of Neurology* **16**(3): 295-304.

Brown, P., Cervenakova, L., Goldfarb, L. G., Mc Combie, W. R., Rubenstein, R., Will, R. G., Pocchiari, M., Martinez-Lage, J. F., Scalici, C., Masullo, C., Graupera, G., Ligan, J. and Gajdusek, D. C. (1994a). Iatrogenic Creutzfeldt-Jakob disease: An example of the interplay between ancient genes and modern medicine. *Neurology* **44**: 291-293.

Brown, P., Gibbs, C. J., Rodgers-Johnson, P., Asher, D., Sulima, M., Bacote, A., Goldfarb, L. and Gajdusek, D. (1994b). Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Annals of Neurology* ***35***(5): 513-29.

Brown, P. (1998a). On the origins of BSE. *Lancet* **352**(9124): 252-253.

Brown, P. and Bradley, R. (1998b). 1755 and all that: a historical primer of transmissible spongiform encephalopathy. *BMJ* **317**(7174): 1688-92.

Brown, P., Cervenakova, L., McShane, L. M., Barber, P., Rubenstein, R. and Drohan, W. N. (1999). Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans [see comments]. *Transfusion* **39**(11-12): 1169-78.

Bruce, M. and Fraser, H. (1982). Focal and asymetrical vacuolar lesions in the brains of mice infected with certain strains of scrapie. *Acta Neuropathol* **58**: 133-140.

Bruce, M., McBride, P. and Facquhar, C. (1989). Precise targeting of the pathology of the sialoglycoprotein PrP, and neuronal vacualization in mouse scrapie. *Neurosci. Lett* **102**: 1-6.

Bruce, M., Chree, A., McConnell, I., Foster, J., Pearson, G. and Fraser, H. (1994). Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **343**(1306): 405-11.

Bruce, M. E., McConnell, I., Fraser, H. and Dickinson, A. G. (1991). The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *Journal of General Virology* **72**(Pt 3): 595-603.

Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H. and Bostock, C. J. (1997). Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389**(6650): 498-501.

BSE Inquiry (2000a). Conclusions drawn from the scientific response to BSE. *http://www.bseinquiry.gov.uk/report/volume1/execsum2.htm* **2: Science**.

BSE Inquiry (2000b). *The Inquiry into BSE and variant CJD in the United Kingdom. Volume 13: Industry Processes and Controls. 4. Mechanically Recovered Meat.* Available at: http://www.bseinquiry.gov.uk/report/volume13/chaptec2.htm.

Bueler, H., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. and Weissmann, C. (1993). Mice devoid of PrP are resistant to scrapie. *Cell* **73**(7): 1339-47.

Canadian Food Inspection Agency Animal Products Animal Health and Production (2001). Chronic Wasting Disease (CWD) of Deer and Elk. *http://www.inspection.gc.ca/english/anima/heasan/disemala/cwdmdce.shtml*.

Caramelli, M., Ru, G., Casalone, C., Bozzetta, E., Acutis, P., Calella, A. and Forloni, G. (2001). Evidence for the
transmission of scrapie to sheep and goats from a vaccine against Mycoplasma agalactiae. *Veterinary Record* **148**(17): 531-6.

Carlson, G. A., DeArmond, S. J., Torchia, M., Westaway, D. and Prusiner, S. B. (1994). Genetics of prion diseases and prion diversity in mice. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **343**(1306): 363-9.

Cervenakova, L., Goldfarb, L. G., Garruto, R., Lee, H. S., Gajdusek, D. C. and Brown, P. (1998). Phenotype-genotype studies in kuru: implications for new variant Creutzfeldt-Jakob disease. *Proceedings of the National Academy of Sciences of the United States of America* **95**(22): 13239-41.

Chesebro, B. (1999). Prion protein and the transmissible spongiform encephalopathy diseases. *Neuron* **24**(3): 503-6.

CJD Surveillance Unit (2001). Information on the new variant of CJD. *http://www.cjd.ed.ac.uk/*.

Clark, W. W., Hourrigan, J. L. and Hadlow, W. J. (1995). Encephalopathy in cattle experimentally infected with the scrapie agent. *American Journal of Veterinary Research* **56**(5): 606-12.

Collinge, J., Palmer, M. and Dryden, A. (1991). Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* **337**(8755): 1441-2.

Collinge, J., Sidle, K. C., Meads, J., Ironside, J. and Hill, A. F. (1996). Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD [see comments]. *Nature* **383**(6602): 685-90.

Collinge, J. and Palmer, M. (1997). Human Prion Diseases. *Prion Diseases*. J. Collinge and M. Palmer, Oxford University Press**:** 18-49.

Cullie, J. and Chelle, P. (1936). La maladie dite tremblante du mouton, est-elle inoculable? *Comptes rendu de l' Academie des Sciences* **203**: 1552-1554.

Cullie, J. and Celle, P.-L. (1939). Transmission experimentale de la tremblante chez la chevre. *Comptes Rendus Academie des Sciences* **208**: 1058-1060.

Cutlip, R., Miller, J., Hamir, A., Peters, J., Robinson, M., Jenny, A., Lehmkuhl, H., Taylor, W. and Bisplinghoff, F. (2001). Resistance of cattle to scrapie by the oral route. *Canadian Journal of Veterinary Research.* **65**(2): 131-132.

Cutlip, R. C., Miller, J. M., Race, R. E., Jenny, A. L., Katz, J. B., Lehmkuhl, H. D., DeBey, B. M. and Robinson, M. M. (1994). Intracerebral transmission of scrapie to cattle. *Journal of Infectious Diseases* **169**(4): 814-20.

Cutlip, R. C., Miller, J. M. and Lehmkuhl, H. D. (1997). Second passage of a US scrapie agent in cattle. *Journal of Comparative Pathology* **117**(3): 271-5.

Dawson, M., Wells, G. A. H., Parker, B. N. J. and Scott, A. (1990). Primary, parenteral transmission of BSE to a pig. *Vet. Rec.* **127**: 338.

de Koeijer, A., Schreuder, B., Heesterbeek, H., Oberthur, R., Wilesmith, J. and de Jong, M. (1999). *BSE Risk Assessment by Calculating the Basic Reproduction Ratio for the Infection Among Cattle*. Lelystad, The Netherlands, Institute for Animal Science and Health.

Det Norske Veritas (1997). *Risk from BSE via environmental pathways*.

Detwiler, L. (1992). Scrapie. *Revue Scientifique et Technique* **11**(2): 491-537.

Detwiler, L. (2000). U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Personal Communication.

Detwiler, L. (2001). U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Personal Communication.

Dickinson, A., Fraser, H. and Outram, G. (1976). Scrapie incubation time can exceed natural lifespan. *Nature* **256**(5520): 732-3.

Dickinson, A. G. and Meikle, V. M. (1971). Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent. *Molecular & General Genetics* **112**(1): 73-9.

Dickinson, A. G. and Outram, G. W. (1988). Genetic aspects of unconventional virus infections: the basis of the virino hypothesis. *Ciba Foundation Symposium* **135**: 63-83.

Diringer, H., Beekes, M. and Oberdieck, U. (1994). The nature of the scrapie agent: the virus theory. *Annals of the New York Academy of Sciences* **724**: 246-58.

Dlouhy, S. R., Hsiao, K., Farlow, M. R., Foroud, T., Conneally, P. M., Johnson, P., Prusiner, S. B., Hodes, M. E. and Ghetti, B. (1992). Linkage of the Indiana kindred of Gerstmann-

Sträussler-Scheinker disease to the prion protein gene. *Nature Genetics* **1**(1): 64-7.

DNV (Det Norske Veritas) (1997). *Assessment of Risk from possible BSE infectivity in dorsal root ganglia*. Report C7831, Rev 1. London, UK.

Doherr, M. G., Heim, D., Vandevelde, M. and Fatzer, R. (1999). Modelling the expected numbers of preclinical and clinical cases of bovine spongiform encephalopathy in Switzerland. *Veterinary Record* **145**(6): 155-60.

Donnelly, C. (1998). Maternal transmission of BSE: Interpretation of the data on the offspring of BSE-affected pedigree suckler cows. *Veterinary Record* **142**(21): 579-580.

Donnelly, C. and Ferguson, N. (2000). *Statistical Aspects of BSE and vCJD*, Chapman & Hall/CRC.

Donnelly, C. A., Ferguson, N. M., Ghani, A. C., Wilesmith, J. W. and Anderson, R. M. (1997a). Analysis of dam-calf pairs of BSE cases: Confirmation of a maternal risk enhancement. *Proceedings of the Royal Society of London - Series B: Biological Sciences* **264**(1388): 1647-56.

Donnelly, C. A., Ferguson, N. M., Ghani, A. C., Woolhouse, M. E., Watt, C. J. and Anderson, R. M. (1997b). The epidemiology of BSE in cattle herds in Great Britain. I. Epidemiological processes, demography of cattle and approaches to control by culling. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **352**(1355): 781-801.

Duguid, J. R., Rohwer, R. G. and Seed, B. (1988). Isolation of cDNAs of scrapie-modulated RNAs by subtractive hybridization of a cDNA library. *Proceedings of the National Academy of Sciences of the United States of America* **85**(15): 5738-42.

Eastern Research Group, I. (1996). TSE Regulatory Options Cost Analysis. *http://www.fda.gov/cvm/index/bse/tse1.pdf*.

Ebringer, A., Thorpe, C., Pirt, J., Wilson, C., Cunningham, P. and Ettelaie, C. (1997). Bovine spongiform encephalopathy: is it an autoimmune disease due to bacteria showing molecular mimicry with brain antigens? *Environmental Health Perspectives* **105**(11): 1172-4.

Elsen, J. M., Amigues, Y., Schelcher, F., Ducrocq, V., Andreoletti, O., Eychenne, F., Khang, J. V., Poivey, J. P., Lantier, F. and Laplanche, J. L. (1999). Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. *Archives of Virology* **144**(3): 431-45.

European Commission (1999a). The evaluation of tests for the diagnosis of Transmissible Spongiform Encephalopathy in Bovines (8 July 1999). *http://europa.eu.int/comm/food/fs/bse/bse12_en.html*.

European Commission (1999b). No evidence for BSE transmission through milk. *http://europa.eu.int/comm/dgs/health_consumer/library/press/press28_en.html*.

European Commission (2000). Commission Decision of 5 June 2000 amending Decision 98/272/EC on epidemio-surveillance for transmissible spongiform encephalopathies (notified under document number C(2000) 1144).

European Commission (2001). Chronological overview of Community legislation concerning BSE. *http://europa.eu.int/comm/food/fs/bse/bse15_en.pdf*.

European Union Scientific Steering Committee (1998a). Opinion on possible links between BSE and Organophosphates used as pesticides against ecto- and endoparasites in cattle - Report and opinion adopted at the Scientific Steering Committee meeting of 25-26 June 1998.

European Union Scientific Steering Committee (1998b). Opinion on the Safety of Gelatine adopted at the Scientific Steering Committee at its plenary meeting of 26-27 March 1998 following a public consultation on the preliminary opinion adopted on 19-20 February 1998 (Version updated on 3.04.98) - Background. *http://europa.eu.int/comm/food/fs/sc/ssc/out09_en.html*.

European Union Scientific Steering Committee (1999a). *Opinion of the Scientific Steering Committee on the Human Exposure Risk (HER) via food with respect to BSE - Adopted on 10 December 1999*. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out67_en.html.

European Union Scientific Steering Committee (1999b). *Report on The Risk Born by Recycling Animal By-Products as Feed with Regard to Propagating TSE's in Non-ruminant Farmed Animals*. Prepared by a Working Group for the Scientific Steering Committee as an input in the elaboration of the opinion on the same subject adopted on 16-17 September 1999. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out59_en.html.

European Union Scientific Steering Committee (2000a). *Opinion - Oral Exposure of Humans to the BSE Agent: Infective Dose and Species Barrier Adopted by the SSC at its Meeting of 13-14 April 2000 Following a Public Consultation via Internet Between 6 and 27 March 2000*. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out79_en.pdf.

European Union Scientific Steering Committee (2000b). *Minutes of the Scientific Steering Committee Meeting of 20-21 January 2000* Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out72_en.html.

European Union Scientific Steering Committee (2000c). *Opinion of the Scientific Steering Committee on a Method for Assessing the Geographical BSE-Risk (GBR) of a Country or Region (update, January 2000)* Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out68_en.pdf.

European Union Scientific Steering Committee (2000d). *Report on the Assessment of the Geographical BSE - Risk of USA (July 2000)*. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out137_en.pdf.

European Union Scientific Steering Committee (2000e). *Opinion on the Safety of Ruminant Blood With Respect to TSE Risks Adopted by the SSC at its Meeting of 13-14 April 2000*. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out74_en.pdf.

Ferguson, N., Donnelly, C., Woolhouse, M. and Anderson, R. (1999). Estimation of the basic reproduction number of BSE: the intensity of transmission in British cattle. *Proceedings of the Royal Society of London - Series B: Biological Sciences.* **266(1414):23-32,**(1414): 23-32.

Ferguson, N. M., Donnelly, C. A., Woolhouse, M. E. and Anderson, R. M. (1997a). A genetic interpretation of heightened risk of BSE in offspring of affected dams. *Proceedings of the Royal Society of London - Series B: Biological Sciences* **264**(1387): 1445-55.

Ferguson, N. M., Donnelly, C. A., Woolhouse, M. E. and Anderson, R. M. (1997b). The epidemiology of BSE in cattle herds in Great Britain. II. Model construction and analysis of transmission dynamics. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **352**(1355): 803-838.

Fitzsimmons, W. M. and Pattison, I. H. (1968). Unsuccesful attempts to transmit scrapie by nematode parasites. *Research in Veterinary Science* **9**: 281-283.

Floyd, L. (2001). Kansas Department of Wildlife and Parks. Personal Communication.

Foster, J., Bruce, M., McConnell, I., Chree, A. and Fraser, H. (1996). Detection of BSE infectivity in brain and spleen of experimentally infected sheep. *Vet Rec.* **138**(22): 546-8.

Foster, J., Parnham, D., Chong, A., Goldmann, W. and N., H. (2001). Clinical signs, histopathology and genetics of
experimental transmission of BSE and natural scrapie to sheep and goats. *Veterinary Record.* **148**(6): 164-71.

Foster, J. D., McKelvey, W. A., Mylne, M. J., Williams, A., Hunter, N., Hope, J. and Fraser, H. (1992). Studies on maternal transmission of scrapie in sheep by embryo transfer. *Veterinary Record* **130**(16): 341-3.

Foster, J. D., Hope, J. and Fraser, H. (1993). Transmission of bovine spongiform encephalopathy to sheep and goats. *Veterinary Record* **133**(14): 339-41.

Franco, D. (2001). Vice President, National Renderers Association. Personal Communication.

Fraser, H. and Dickinson, A. (1968). The sequential development of brain lesions of scrapie in three strains of mice. *J. Comp. Pathol.* **78**: 301-311.

FSIS Directive 7160.2 (1997). “Meat” prepared using advanced mechanical meat/bone separation machinery and meat recovery systems. *http://www.fsis.usda.gov/oppde/rdad/fsisdirectives/fsisdir7160%2D2.pdf*.

Gajdusek, D. C., Gibbs, C. J. and Alpers, M. (1966). Experimental transmission of a Kuru-like syndrome to chimpanzees. *Nature* **209**(25): 794-6.

Gale, P. and Stanfield, G. (2001). Towards a quantitative risk assessment for BSE in sewage sludge. *Journal of Applied Microbiology* **91**(3): 563-569.

Garland, T., Bauer, N. and Bailey, M., Jr. (1996). Brain emboli in the lungs of cattle after stunning [letter] [see comments]. *Lancet* **348**(9027): 610.

Gibbs, C. J., Gajdusek, C. J. and Amyx (1979). Strain variation in the viruses of Creutzfeldt-Jakob disease and kuru. *Slow Transmissible Diseases of the Nervous System*. S. Prusiner and W. Hadlow, Academic Press, New York. **2:** 87-110.

Gibbs, C. J., Jr., Safar, J., Ceroni, M., Di Martino, A., Clark, W. W. and Hourrigan, J. L. (1990). Experimental transmission of scrapie to cattle. *Lancet* **335**(8700): 1275.

Glatzel, M. and Aguzzi, A. (2001). The shifting biology of the prions. *Brain Research Reviews* **In press**.

Goldmann, W., Hunter, N., Martin, T., Dawson, M. and Hope, J. (1991). Different forms of the bovine PrP gene have five or six copies of a short, G-C-rich element within the protein-coding exon. *Journal of General Virology* **72**(Pt 1): 201-4.

Goldmann, W., Hunter, N., Somerville, R. and Hope, J. (1996). Prion phylogeny revisited. *Nature* **382**(6586): 32-3.

Gordon, W. S. (1939). *Studies of louping-ill, tick borne fever and scrapie*. 3rd International Congress for Microbiology.

Gordon, W. S. (1946). Louping ill, tickborne fever and scrapie. *Veterinary Record* **58**: 516-525.

Gordon, W. S. (1959). *Scrapie Panel*. Proceedings of 63rd Annual Meeting of the US LivestockSanitary Association.

Gould, D. (2000). *Geographically Targeted Survey of Cattle in Northeast Colorado for Evidence of Chronic Wasting Disease (CWD)*. United States Animal Health Association.

Griebel, P. J. and Hein, W. R. (1996). Expanding the role of Peyer's patches in B-cell ontogeny. *Immunology Today* **17**(1): 30-9.

Hadlow, W., Race, R. and RC, K. (1987). Experimental Infection of sheep and goats with transmissible mink spongiform encephalopathy virus. *Canadian Journal of Veterinary Research* **51**: 135-144.

Hadlow, W. J. (1959). Scrapie and kuru. *Lancet*(ii): 289-290.

Hadlow, W. J., Kennedy, R. C., Race, R. E. and Eklund, C. M. (1980). Virologic and neurohistologic findings in dairy goats affected with natural scrapie. *Veterinary Pathology* **17**(2): 187-99.

Hadlow, W. J., Kennedy, R. C. and Race, R. E. (1982). Natural infection of Suffolk sheep with scrapie virus. *Journal of Infectious Diseases* **146**(5): 657-64.

Hamir, A., Cutlip, R., Miller, J., Williams, E., Stack, M., Miller, M., O'Rourke, K. and Chaplin, M. (2001). Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle. *J Vet Diagn Invest.* **13**(1): 91-96.

Hansen, M. (1999). Creutzfeldt-Jakob disease. *New England Journal of Medicine.* **340**(21): 1689.

Harbour, D. (2001). *Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep*. School of Medicine, University of Bristol. Available at: http://europa.eu.int/comm/research/press/1998/pr2710en.html.

Hartsough, G. R. and Burger, D. (1965). Encephalopathy of the mink. I . Epizootiologic and clinical observations. *Journal of Infectious Diseases* **115**: 387-392.

Heim, D. (2001). Swiss Federal Veterinary Office. Personal Communication.

Hill, A. F., Desbruslais, M., Joiner, S., Sidle, K. C., Gowland, I., Collinge, J., Doey, L. J. and Lantos, P. (1997). The same prion strain causes vCJD and BSE. *Nature* **389**(6650): 448-50.

Hill, A. F., Antoniou, M. and Collinge, J. (1999). Protease-resistant prion protein produced in vitro lacks detectable infectivity. *Journal of General Virology* **80**(Pt 1): 11-4.

Hill, A. F., Joiner, S., Linehan, J., Desbruslais, M., Lantos, P. L. and Collinge, J. (2000). Species-barrier-independent prion replication in apparently resistant species. *Proceedings of the National Academy of Sciences of the United States of America* **97**(18): 10248-53.

Hoinville, L., McLean, A. R., Hoek, A., Gravenor, M. B. and Wilesmith, J. (1999). Scrapie occurrence in Great Britain. *Veterinary Record* **145**(14): 405-6.

Hoinville, L. J. (1994). Decline in the incidence of BSE in cattle born after the introduction of the 'Feed Ban'. *Veterinary Record* **134**: 274-275.

Hoinville, L. J. (1996). A review of the epidemiology of scrapie in sheep. *Revue Scientifique et Technique* **15**(3): 827-52.

Holman, R. C., Khan, A. S., Kent, J., Strine, T. W. and Schonberger, L. B. (1995). Epidemiology of Creutzfeldt-Jakob disease in the United States, 1979-1990: analysis of national mortality data. *Neuroepidemiology* **14**(4): 174-81.

Horn, G., Bobrow, M., Bruce, M., Goedhert, M., McLean, A. and Webster, J. (2001). *Review of the Origin of BSE*. UK Department for Environment, Food, and Rural Affairs. Accessed: 2002. Available at: http://www.defra.gov.uk/animalh/bse/bseorigin.pdf.

Hourrigan, J., Klingsporn, A., Clark, W. W. and DeCamp, M. (1979). *Slow transmissible diseases of the central nervous system*, Academy Press, Nwe York.

Hsiao, K. and Prusiner, S. (1990). Inherited human prion diseases. *Neurology* **40**: 1820-1827.

Hsiao, K., Scott, M., Foster, D., DeArmond, S. J., Groth, D., Serban, H. and Prusiner, S. B. (1991). Spontaneous neurodegeneration in transgenic mice with prion protein codon 101 proline----leucine substitution. *Annals of the New York Academy of Sciences* **640**: 166-70.

Hsiao, K. K., Groth, D., Scott, M., Yang, S. L., Serban, H., Rapp, D., Foster, D., Torchia, M., Dearmond, S. J. and Prusiner, S. B. (1994). Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein. *Proceedings of the National Academy of Sciences of the United States of America* **91**(19): 9126-30.

Hueston, W. (1997). 62 FR 551-583: Substances Prohibited from Use in Animal Food or Feed; Animal Proteins Prohibited in Ruminant Feed; Proposed Rule. 1997.

Hunter, N., Foster, J. D. and Hope, J. (1992). Natural scrapie in British sheep: breeds, ages and PrP gene polymorphisms. *Veterinary Record* **130**(18): 389-92.

Hunter, N., Goldmann, W., Benson, G., Foster, J. D. and Hope, J. (1993). Swaledale sheep affected by natural scrapie differ significantly in PrP genotype frequencies from healthy sheep and those selected for reduced incidence of scrapie. *Journal of General Virology* **74**(Pt 6): 1025-31.

Hunter, N., Goldmann, W., Smith, G. and Hope, J. (1994). Frequencies of PrP gene variants in healthy cattle and cattle with BSE in Scotland. *Veterinary Record* **135**(17): 400-3.

Hunter, N., Foster, J. D., Goldmann, W., Stear, M. J., Hope, J. and Bostock, C. (1996). Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. *Archives of Virology* **141**(5): 809-24.

Hunter, N. (1997a). PrP genetics in sheep and the applications for scrapie and BSE. *Trends in Microbiology* **5**(8): 331-4.

Hunter, N., Goldmann, W., Foster, J. D., Cairns, D. and Smith, G. (1997b). Natural scrapie and PrP genotype: case-control studies in British sheep. *Veterinary Record* **141**(6): 137-40.

Hunter, N., Moore, L., Hosie, B., Dingwall, W. and Greig, A. (1997c). Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. *Veterinary Record* **140**(3): 59-63.

Hunter, N. (1998a). Scrapie. *Molecular Biotechnology* **9**: 225-234.

Hunter, N. and Cairns, D. (1998b). Scrapie-free Merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible PrP genotypes. *Journal of General Virology* **79**(Pt 8): 2079-82.

Ikeda, T., Horiuchi, M., Ishiguro, N., Muramatsu, Y., Kai-Uwe, G. and Shinagawa, M. (1995). Amino acid polymorphisms of PrP with reference to onset of scrapie in Suffolk and Corriedale sheep in Japan. *Journal of General Virology* **76**(10): 2577-81.

Ironside, J. W., Head, M. W., Bell, J. E., McCardle, L. and Will, R. G. (2000). Laboratory diagnosis of variant Creutzfeldt-Jakob disease. *Histopathology* **37**(1): 1-9.

Kelley, L. C., Hafner, S., McCaskey, P. C., Sutton, M. T. and Langheinrich, K. A. (2000). An evaluation of methods for the detection of spinal cord in product derived from advanced meat recovery systems. *Journal of Food Protection* **63**(8): 1107-12.

Kelly, D., Rearson, H., Wright, A. and Greenham, L. (1980). Morbidity in captive white tigers. *Comparative Pathology of Zoo animals*. R. Montali and G. Migaki. Washington DC, Institute Press**:** 183-188.

Kimberlin, R., Cole, S. and Walker, C. (1987). Temporary and permanent modifications to a single strain of mouse scrapie on transmission to rats and hamsters. *Journal of General Virology. 68 (Pt 7):1875-81* **68**(7): 1875-81.

Kimberlin, R. H., Walker, C. A., Millson, G. C., Taylor, D. M., Robertson, P. A., Tomlinson, A. H. and Dickinson, A. G. (1983). Disinfection studies with two strains of mouse-passaged scrapie agent. Guidelines for Creutzfeldt-Jakob and related agents. *Journal of the Neurological Sciences* **59**(3): 355-69.

Kimberlin, R. H. and Walker, C. A. (1988). *Pathogenesis of experimental scrapie*. Novel infectious agents and the central nervous system-Ciba Foundation Symposium, Wiley, Chichester.

Kimberlin, R. H. and Walker, C. A. (1989). Pathogenesis of scrapie in mice after intragastric infection. *Virus Research* **12**(3): 213-20.

Kimberlin, R. H. (1990). Transmissible encephalopathies in animals. *Canadian Journal of Veterinary Research* **54**(1): 30-7.

Kimberlin, R. H. and Wilesmith, J. W. (1994). Bovine spongiform encephalopathy. Epidemiology, low dose exposure and risks. *Annals of the New York Academy of Sciences* **724**: 210-20.

Klatzo, I., Gajdusek, D. C. and Zigas, V. (1957). Pathology of kuru. *Laboratory Investigations* **8**: 799-847.

Laplanche, J., Chatelain, J., Westaway, D., Thomas, S., Dussaucy, M., Brugere-Picoux, J. and Launay, J. (1993). PrP polymorphisms associated with natural scrapie discovered by denaturing gradient gel electrophoresis. *Genomics* **15**(1): 30-7.

Lasmezas, C. I., Deslys, J. P., Robain, O., Jaegly, A., Beringue, V., Peyrin, J. M., Fournier, J. G., Hauw, J. J., Rossier, J. and Dormont, D. (1997). Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. *Science* **275**(5298): 402-5.

Lord Phillips, o. W. M., Bridgeman, J. C. and Ferguson-Smith, M. F. (2000). The BSE Inquiry.

Lucker, E., Eigenbrodt, E., Wenisch, S., Leiser, R. and Bulte, M. (2000). Identification of central nervous system tissue in retail meat products. *J Food Prot.* **63**(2): 258-63.

MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) (2000). *BSE: Measures Taken by the UK: Report for the Month to the End of August 2000*. Available at: http://www.defra.gov.uk/animalh/bse/bse-publications/monrep/monrep29.pdf.

MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) (2001a). *BSE information: Transmission of BSE (Infectivity in Tissues)*. Accessed: 2003. Available at: http://www.defra.gov.uk/animalh/bse/bse-science/level-4-transmis.html#infect.

MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) (2001b). *BSE information: Transmission of BSE (Infectivity to Other Species)*.

Available at: http://www.defra.gov.uk/animalh/bse/bse-science/level-4-transmis.html#species.

MAFF (Ministry of Agriculture Fisheries and Food - now Dept for Environment Food and Rural Affairs) (2001c). *BSE Information: Specified Risk Material*. Available at: http://www.defra.gov.uk/animalh/bse/public-health/level-3-srms.html.

Manson, J. C., Jamieson, E., Baybutt, H., Tuzi, N. L., Barron, R., McConnell, I., Somerville, R., Ironside, J., Will, R., Sy, M. S., Melton, D. W., Hope, J. and Bostock, C. (1999). A single amino acid alteration (101L) introduced into murine PrP dramatically alters incubation time of transmissible spongiform encephalopathy. *EMBO Journal* **18**(23): 6855-64.

Manuelidis, L., Sklaviadis, T., Akowitz, A. and Fritch, W. (1995). Viral particles are required for infection in neurodegenerative Creutzfeldt-Jakob disease. *Proceedings of the National Academy of Sciences of the United States of America* **92**(11): 5124-8.

Marsh, R. F., Burger, D., Eckroade, R., ZuRhein, G. M. and Hanson, R. P. (1969). A preliminary report on the experimental host range of transmissible mink encephalopathy agent. *J. Inf. Dis.* **120**: 713-719.

Marsh, R. F., Bessen, R. A., Lehmann, S. and Hartsough, G. R. (1991). Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. *Journal of General Virology* **72**(Pt 3): 589-94.

Masters, C. L., Harris, J., Gajdusek, C., Gibbs, C., Bernoulli, C. and Asher, D. M. (1978). Creutzfeldt-Jakob Disease: Patterns of Wordwide occurrence and the Significance of Familial and Sporadic Clustering. *Ann Neurol* **5**: 177-188.

McKenzie, D., Bartz, J., Mirwald, J., Olander, D., Marsh, R. and Aiken, J. (1998). Reversibility of scrapie inactivation is enhanced by copper. *Journal of Biological Chemistry* **273**(40): 25545-7.

McKinley, M. P., Bolton, D. C. and Prusiner, S. B. (1983). A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**(1): 57-62.

McLean, C. A., Ironside, J. W., Alpers, M. P., Brown, P. W., Cervenakova, L., Anderson, R. M. and Masters, C. (1998). Comparative Neuropathology of Kuru with New Variant Creutzfeldt Jakob Disease: Evidence of Strain of Agent Predominating over Genotype of host. *Brain Pathology* **8**: 429-437.

Miller, M., Williams, S., McCarty, C., Spraker, T., Kreeger, T., Larsen, T. and Thorne, E. (2000). Epizootiology of Chronic Wasting Disease in free-ranging cervids in Colorado and Wyaoming. *Jouranl of Wildlife Diseases* **36**(4): 676-690.

Miller, M. W., Wild, M. A. and Williams, E. S. (1998). Epidemiology of chronic wasting disease in captive Rocky Mountain elk. *Journal of Wildlife Diseases* **34**(3): 532-8.

Nathanson, N., Wilesmith, J. and Griot, C. (1997). Bovine spongiform encephalopathy (BSE): causes and consequences of a common source epidemic. *American Journal of Epidemiology* **145**(11): 959-69.

Nowak, R., Ronald and Paraiso, J. (1983). *Walker's Mammals of the World*, Johns Hopkins Univ. Press.

OIE (2000). Bovine spongiform encephalopathy. *http://www.oie.int/eng/info/en_esb.htm*.

OIE (2001). Surveillance and Monitoring of Bovine Spongiform Encephaolopathy. *http://www.oie.int/eng/normes/mcode/A_00154.htm*.

O'Rourke, K. I., Besser, T. E., Miller, M. W., Cline, T. F., Spraker, T. R., Jenny, A. L., Wild, M. A., Zebarth, G. L. and Williams, E. S. (1999). PrP genotypes of captive and free-ranging Rocky Mountain elk (Cervus elaphus nelsoni) with chronic wasting disease. *Journal of General Virology* **80**(Pt 10): 2765-9.

Palmer, M. S., Dryden, A. J., Hughes, J. T. and Collinge, J. (1991). Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. [see comments]. [erratum appears in Nature 1991 Aug 8;352(6335):547]. *Nature* **352**(6333): 340-2.

Parchi, P., Giese, A., Capellari, S., Brown, P., Schulz-Schaeffer, W., Windl, O., Zerr, I., Budka, H., Kopp, N., Piccardo, P., Poser, S., Rojiani, A., Streichemberger, N., Julien, J., Vital, C., Ghetti, B., Gambetti, P. and Kretzschmar, H. (1999). Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Annals of Neurology* **46**(2): 224-33.

Pattison, I., Gordon, W. and Millson, G. (1959). The possible natural transmission of scrapie in goats. *Journal of Comparative Pathology* **71**: 101-108.

Platt, T. (2001). Executive Director, Fur Commission USA. Personal Communication.

Poulter, M., Baker, H. F., Frith, C. D., Leach, M., Lofthouse, R., Ridley, R. M., Shah, T., Owen, F., Collinge, J. and Brown, J. (1992). Inherited prion disease with 144 base pair gene insertion. 1. Genealogical and molecular studies. *Brain* **115**(Pt 3): 675-85.

Priola, S. A., Caughey, B., Race, R. E. and Chesebro, B. (1994). Heterologous PrP molecules interfere with accumulation of protease-resistant PrP in scrapie-infected murine neuroblastoma cells. *Journal of Virology* **68**(8): 4873-8.

ProMED-mail (2001, April 18). BSE trigger suspected to be African antelope.

Prusiner, S. (1989). Scrapie Prions. *Annu. Rev. Microbiol.* **43**: 345-74.

Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science* **216**(4542): 136-44.

Prusiner, S. B. (1994). Biology and genetics of prion diseases. *Annual Review of Microbiology* **48**: 655-86.

Prusiner, S. B. (1998). Prions. *Proceedings of the National Academy of Sciences of the United States of America* **95**(23): 13363-83.

Public Citizen (2001). Letter to the USDA and FDA Re: BSE.

Purdey, M. (1996). The UK epidemic of BSE: slow virus or chronic pesticide-initiated modification of the prion protein? Part 2: An epidemiological perspective. *Medical Hypotheses* **46**(5): 445-54.

Race, R. and Chesebro, B. (1998a). Scrapie infectivity found in resistant species. *Nature* **392**(6678): 770.

Race, R., Jenny, A. and Sutton, D. (1998b). **Scrapie** infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis. *Journal of Infectious Diseases* **178**(4): 949-53.

Raymond, G., Hope, J., Kocisko, D., Priola, S., Raymond, L., Bossers, A., Ironside, J., Will, R., Chen, S., Petersen, R., Gambetti, P., Rubenstein, R., Smits, M., Lansbury, P. and Caughey, B. (1997). Molecular assessment of the potential transmissibilities of BSE and scrapie to humans. *Nature* **388**(6639): 285-8.

Raymond, G., Bossers, A., Raymond, L., O'Rourke, K., McHolland, L., Bryant, P., Miller, M., Williams, E., Smits, M. and Caughey, B. (2000). Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. *EMBO* **19**(17): 4425-30.

Rehbinder, C. and Petersson, L. (1994). Cerebellar abiotrophy in a moose (Alces alces L) related to copper deficiency. A case report. *Acta Veterinaria Scandinavica* **35**(1): 103-6.

Ridley, R. and Baker, H. (1996). The myth of maternal transmission of spongiform encephalopathy. *BMJ* **311**(7012): 1071-5.

Robinson, M. M., Hadlow, W. J., Knowles, D. P., Huff, T. P., Lacy, P. A., Marsh, R. F. and Gorham, J. R. (1995). Experimental infection of cattle with the agents of transmissible mink encephalopathy and scrapie. *Journal of Comparative Pathology* **113**(3): 241-51.

Rocky Mountain Elk Foundation (1997). Status of the Elk im North America 1975-1995.

Romans, J. and Ziegler, P. (1974). *The meat we eat*. Danville, Illinois, The Interestate Printers & Publishers Inc,.

Rudbeck, J. (1999). More Products Shipping Overseas but Cost Less. *Renderer, The National Magazine of the Renderering*(October).

Ryder, S. J., Hawkins, S. A., Dawson, M. and Wells, G. A. (2000). The neuropathology of experimental bovine spongiform encephalopathy in the pig. *Journal of Comparative Pathology* **122**(2-3): 131-43.

Schaller, O., Fatzer, R., Stack, M., Clark, J., Cooley, W., Biffiger, K., Egli, S., Doherr, M., Vandevelde, M., Heim, D., Oesch, B. and Moser, M. (1999). Validation of a western immunoblotting procedure for bovine PrP(Sc) detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE). *Acta Neuropathologica* **98**(5): 437-43.

Schmidt, G., Yemm, R. S., Childs, K. D., O"Callagham, J. P. and Hossner, K. L. (2001). Beta site analysis and verification of different glial fibrillary acidic protein (GFAP) analyses as

accurate detectors of central nervous system tissue in advanced meat recovery (AMR) products. *In press*.

Schmidt, G. R., Hossner, K. L., Yemm, R. S., Gould, D. H. and O'Callaghan, J. P. (1999). An enzyme-linked immunosorbent assay for glial fibrillary acidic protein as an indicator of the presence of brain or spinal cord in meat. *Journal of Food Protection* **62**(4): 394-7.

Schreuder, B. C., Wilesmith, J., Ryan, J. B. M. and Straub, O. C. (1997). Risk of BSE from the import of cattle from UK into countries in the European Union. *Veterinary Record* **141**: 187-190.

Schreuder, B. E., Geertsma, R. E., van Keulen, L. J., van Asten, J. A., Enthoven, P., Oberthur, R. C., de Koeijer, A. A. and Osterhaus, A. D. (1998). Studies on the efficacy of hyperbaric rendering procedures in inactivating bovine spongiform encephalopathy (BSE) and scrapie agents. *Veterinary Record* **142**(18): 474-80.

Scott, M. R., Will, R., Ironside, J., Nguyen, H. O., Tremblay, P., DeArmond, S. J. and Prusiner, S. B. (1999). Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proceedings of the National Academy of Sciences of the United States of America* **96**(26): 15137-42.

Sigurdson, C. J., Williams, E. S., Miller, M. W., Spraker, T. R., O'Rourke, K. I. and Hoover, E. A. (1999). Oral transmission and early lymphoid tropism of chronic wasting disease PrPres in mule deer fawns (Odocoileus hemionus). *Journal of General Virology* **80**(Pt 10): 2757-64.

Sigurdson, S. (1991). Epidemiology of scrapie in Iceland with control measures. *Sub acute spongiform encephalopathies. Proceedings of an EC seminar, 12-14 November 1990***:** 233-242.

Simmons, M. M., Ryder, S. J., Chaplin, M. C., Spencer, Y. I., Webb, C. R., Hoinville, L. J., Ryan, J., Stack, M. J., Wells, G. A. and Wilesmith, J. W. (2000). Scrapie surveillance in Great Britain: results of an abattoir survey, 1997/98. *Veterinary Record* **146**(14): 391-5.

Skarphedinsson, S., Johannsdottir, R., Gudmundsson, P., Sigurdarson, S. and Georgsson, G. (1994). PrPsc in Icelandic sheep naturally infected with scrapie. *Ann N Y Acad Sci* **724**: 304-9.

Southern States Cooperative (2001). Prices.

Sparks Companies, I. (1999). *Advanced Meat Recovery Systems - An Economic Analysis of the Proposed USDA Regulation*. McLean, VA Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/98-027R/SparksCo_AMR_Economic_Analysis.pdf.

Spongiform Encephalopathy Advisory Committee (1997). Public summary of meeting on 24 October 1997.

Spongiform Encephalopathy Advisory Committee (1999a). *SEAC Annual Report 1997-1998* Available at: http://www.seac.gov.uk/seacrept.pdf.

Spongiform Encephalopathy Advisory Committee (1999b). *SEAC Annual Report 1998-1999* Available at: http://www.seac.gov.uk/98-9rep.pdf.

Spongiform Encephalopathy Advisory Committee (2000). *Risk Assessment for the disposal of treated rendering plant ruminant condensate to agricultural land.*

Stockman, S. (1913). Scrapie: An obscure disease of sheep. *Journal of Comparative Pathology*.

Stringer, S. M., Hunter, N. and Woolhouse, M. E. (1998). A mathematical model of the dynamics of scrapie in a sheep flock. *Mathematical Biosciences* **153**(2): 79-98.

Swiss Federal Veterinary Service (2002). Memo (March 18) to the Harvard Center for Risk Analysis: "Comments on the Harvard study: Evaluation of the potential for Bovine Spongiform Encephalopathy in the United States".

Taylor, D. M. (1989). Scrapie agent decontamination: implications for bovine spongiform encephalopathy. *Veterinary Record* **124**(12): 291-2.

Taylor, D. M. (1991a). Inactivation of the unconventional agents of scrapie, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. *Journal of Hospital Infection* **18 Suppl A**: 141-6.

Taylor, D. M. (1991b). Inactivation of BSE agent. *Developments in Biological Standardization* **75**: 97-102.

Taylor, D. M. (1993). Inactivation of SE agents. *British Medical Bulletin* **49**(4): 810-21.

Taylor, D. M., Woodgate, S. L. and Atkinson, M. J. (1995). Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *Veterinary Record* **137**(24): 605-610.

Taylor, D. M. and Woodgate, S. L. (1997a). Bovine spongiform encephalopathy: the causal role of ruminant-derived protein in cattle diets. *Revue Scientifique et Technique* **16**(1): 187-98.

Taylor, D. M., Woodgate, S. L., Fleetwood, A. J. and Cawthorne, R. J. (1997b). Effect of rendering procedures on the scrapie agent. *Veterinary Record* **141**(25): 643-649.

Tegtmeier, C., Agerholm, J., Bille-Hansen, V., Schaap, P. and Ryder, S. (2001). Firs confirmed native case of bovine spongiform encephalopathy in Denmark. *Vet Rec* **148**: 51-52.

Telling, G. C., Scott, M., Mastrianni, J., Gabizon, R., Torchia, M., Cohen, F. E., DeArmond, S. J. and Prusiner, S. B. (1995). Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**(1): 79-90.

Thornton, I. and Webb, J. S. (1979). Geochemistry and health in the United Kingdom. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **288**(1026): 151-68.

Tiwana, H., Wilson, C., Pirt, J., Cartmell, W. and Ebringer, A. (1999). Autoantibodies to brain components and antibodies to Acinetobacter calcoaceticus are present in bovine spongiform encephalopathy. *Infection & Immunity* **67**(12): 6591-5.

U.S. Department of Agriculture (1997). *Livestock and Carcass Disposition Review*. Food Safety Inspection Service.

U.S. Department of Agriculture (1998). *Animal Disposition Reporting System (ADRS) Livestock Slaughtered in USDA Establishments: Calendar Year 1998*. Food Safety Inspection Service. Accessed: 2001. Available at: http://www.fsis.usda.gov/OPHS/adrsdata/1998adrs/98crm1.htm.

U.S. Department of Agriculture (2000). *Scrapie Project Final Rule, 9 CFR Parts 54 and 79, Docket No. 99-067-2*. Animal Plant Health and Inspection Service. Accessed: 2000. Available at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2000_register&docid=fr27jn00-3.

U.S. Department of Agriculture (2001a). *Factsheet: Chronic Wasting Disease Funding*. Animal and Plant Health Inspection Services. Available at: http://www.aphis.usda.gov/oa/pubs/cwdf.pdf.

U.S. Department of Agriculture (2001b). *Mink Annual Report*. NASS.

U.S. Department of Agriculture (2002). *Bovine Spongiform Encephalopathy: Surveillance*. Animal and Plant Health Inspection Services. Accessed: 2003. Available at: http://www.aphis.usda.gov/lpa/issues/bse/bse-surveillance.html.

U.S. Department of Agriculture (2003). *USDA Actions to Prevent Bovine Spongiform Encephelopathy*. Animal Plant Health Inspection Service. Accessed: 2003. Available at: http://www.aphis.usda.gov/lpa/issues/bse/bsechron.html.

U.S. Department of Agriculture (FSIS) (2002). *Revised Directive for Advanced Meat Recovery Systems*. Accessed: 2003. Available at: http://www.fsis.usda.gov/OA/background/amrdirec.htm.

U.S. Food and Drug Administration (1997). Substances prohibited from use in animal food or feed; Animal proteins prohibited in ruminant feed; Final Rule - 21 CFR Part 589. *Federal Register* **62**(108): 30935.

U.S. Food and Drug Administration (2001a). *Transmisible Spongiform Encephalopathy Advisory Meeting, March 19, 2001*. Available at: http://www.fda.gov/ohrms/dockets/ac/cber01.htm#Transmissible.

U.S. Food and Drug Administration (2001b). *January 10: Update on Ruminant Feed (BSE) Enforcement Activities*. Center for Veterinary Medicine. Accessed: 2001. Available at: http://www.fda.gov/cvm/index/updates/bseup.htm.

UK CJD Surveillance Unit (2003). *The UK Creutzfeldt-Jakob Disease Surveillance Unit*. Accessed: 2003. Available at: http://www.cjd.ed.ac.uk.

USDA-APHIS, V. S. (2000). Comments submitted by US on the "draft report on the assessment of the Geographical BSE-risk of the USA.

Venter, A. (2001). Mad deer in Canadian wild? *Trends in Microbiology* **9**(7): 312.

Vossen, P., Kreysa, J. and Goll, M. (2003). *Overview of the BSE Risk Assessments of the European Commission's Scientific Steering Committee (SSC) and its TSE/BSE ad hoc Group: Adopted Between September 1997 and April 2003*. Available at: http://europa.eu.int/comm/food/fs/sc/ssc/out364_en.pdf.

Walker, K. D., Hueston, W. D., Hurd, H. S. and Wilesmith, J. W. (1991). Comparison of bovine spongiform encephalopathy risk factors in the United States and Great Britain. *Journal of the American Veterinary Medical Association* **199**(11): 1554-61.

Warren, H. V. (1974). Proceedings: Environmental lead: a survey of its possible physiological significance. *Journal of Biosocial Science* **6**(2): 223-38.

Webb, C., Wilesmith, J., Simmons, M. and Hoinville, L. (2001). A stochastic model to estimate the prevalence of scrapie in Great Britain using the results of an abatoir based survey. *Preventive Veterinary Medicine* **51**: 269-287.

Weissmann, C. and Aguzzi, A. (1999). Perspectives: neurobiology. PrP's double causes trouble. *Science* **286**(5441): 914-5.

Wells, G. A., Wilesmith, J. W. and McGill, I. S. (1991). Bovine spongiform encephalopathy: a neuropathological perspective. *Brain Pathology* **1**(2): 69-78.

Wells, G. A., Hawkins, S. A., Green, R. B., Austin, A. R., Dexter, I., Spencer, Y. I., Chaplin, M. J., Stack, M. J. and Dawson, M. (1998). Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Veterinary Record* **142**(5): 103-106.

Wells, G. A., Hawkins, S. A., Green, R. B., Spencer, Y. I., Dexter, I. and Dawson, M. (1999). Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE). *Veterinary Record* **144**(11): 292-294.

Westaway, D., Zuliani, V., Cooper, C., DaCosta, M., Neuman, S., Jenny, A., Detwiler, L. and Prusiner, S. (1994). Homozigocity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. *Genes and Development* **8**: 959-969.

WHO (2001). Fact Sheet: Bovine Spongiform Encephalopathy. *http://www.who.int/inf-fs/en/fact113.html*.

Wilesmith, J. and Ryan, J. B. M. (1992a). Bovine spongiform encephalopathy: Recent observations on the age-specific incidences. *Veterinary Record* **130**: 491-492.

Wilesmith, J. and Ryan, J. B. M. (1993). Bovine spongiform encephelopathy: Observations on the incidence during 1992. *Veterinary Record* **132**: 300-301.

Wilesmith, J. W., Wells, G., Cranwell, M. P. and Ryan, J. B. M. (1988). Bovine spongiform encephelopathy: Epidemiological studies. *Veterinary Record* **123**: 638-644.

Wilesmith, J. W., Ryan, J. B. and Atkinson, M. J. (1991). Bovine spongiform encephalopathy: Epidemiological studies on the origin. *Veterinary Record* **128**(9): 199-203.

Wilesmith, J. W., Ryan, J. B., Hueston, W. D. and Hoinville, L. J. (1992b). Bovine spongiform encephalopathy: epidemiological features 1985 to 1990. *Veterinary Record* **130**(5): 90-4.

Wilesmith, J. W. (1994). An epidemiologist's view of bovine spongiform encephalopathy. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* **343**(1306): 357-61.

Wilesmith, J. W., Wells, G. A., Ryan, J. B., Gavier-Widen, D. and Simmons, M. M. (1997). A cohort study to examine maternally-associated risk factors for bovine spongiform encephalopathy. *Veterinary Record* **141**(10): 239-43.

Will, R., Matthews, W., Smith, P. and Hudson, C. (1986). A retrospective study of Creutzfeldt Jakob Disease in England and Wales 1970-1979. *Epidemilogy J. Neurol. Neurosurg. Psychiatry* **49**: 749-755.

Will, R. G., Ironside, J. W., Zeidler, M., Cousens, S. N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., Hofman, A. and Smith, P. G. (1996). A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**(9006): 921-5.

Will, R. G., Zeidler, M., Stewart, G. E., Macleod, M. A., Ironside, J. W., Cousens, S. N., Mackenzie, J., Estibeiro, K., Green, A. J. and Knight, R. S. (2000). Diagnosis of new variant Creutzfeldt-Jakob disease. *Annals of Neurology* **47**(5): 575-82.

Williams, E. S. and Young, S. (1980). Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *Journal of Wildlife Diseases* **16**(1): 89-98.

Williams, E. S. and Young, S. (1982). Spongiform encephalopathy of Rocky Mountain elk. *Journal of Wildlife Diseases* **18**(4): 465-71.

Williams, E. S. (2001). Department of Veterinary Sciences, University of Wyoming, Laramie. Personal Communication.

Wineland, N. E., Detwiler, L. A. and Salman, M. D. (1998). Epidemiologic analysis of reported scrapie in sheep in the United States: 1,117 cases (1947-1992). *Journal of the American Veterinary Medical Association* **212**(5): 713-8.

Woolhouse, M. E. and Anderson, R. M. (1997). Understanding the epidemiology of BSE. *Trends in Microbiology* **5**(11): 421-4.

Woolhouse, M. E., Stringer, S. M., Matthews, L., Hunter, N. and Anderson, R. M. (1998). Epidemiology and control of scrapie within a sheep flock. *Proceedings of the Royal Society of London - Series B: Biological Sciences* **265**(1402): 1205-10.

Zigas, V. and Gajdusek, D. C. (1957). Kuru: clinical study of a new syndrome resmbling paralysis agitans in native of the Eastern Higlands of Australian New Guinea. *Medical Journal of Australia* **2**: 745-754.