TSE THINK TANK Executive Summary

Transmissible Spongiform Encephalopathy Think Tank Executive Summary

Introduction

First discovered in 1986 in the United Kingdom (U.K.), bovine spongiform encephalopathy (BSE, or Mad Cow disease) has become one of the largest challenges that the U.S. cattle industry has faced. While the U.S. government and beef industry have taken aggressive steps to ensure that this degenerative neurological disease of cattle does not have widespread negative effects on human and animal health, the perceived risks of the disease have complicated international trade relationships and created significant economic issues for U.S. cattle producers. BSE has cost U.S. beef producers more than $5 billion[1] in lost export market access, not to mention the significant resources that have been devoted to correct misperceptions about the disease and its potential implications.

The relatively recent discovery of BSE has meant that there are still several unknowns about this disease. In an effort to better understand BSE and other transmissible spongiform encephalopathies, the National Cattlemen's Beef Association (NCBA) on behalf of the Federation of State Beef Councils organized a Transmissible Spongiform Encephalopathy (TSE) Think Tank, Nov. 1-3, 2006, to summarize what is currently known and identify future research initiatives. Beef industry leaders, federal researchers and disease experts convened to discuss the similarities and differences in worldwide BSE cases, as well as current interventions and controls in place to ensure continued risk reduction of BSE in the United States.

The Checkoff-funded BSE Working Group met in 2000 and established a list of research priorities that have served as a roadmap for TSE science research. However, the new knowledge surrounding TSE diseases as well as additional cases of BSE in North America lead to the need for updated research goals. The TSE Think Tank was organized to bring together a group of researchers and industry leaders to identify new research priorities.

Background

Bovine spongiform encephalopathy is a neurological disease of cattle that affects the central nervous system (CNS). The first cases were detected in the United Kingdom in 1986. The incidence of BSE in the U.K. peaked in 1992 and to date there have been more than 190,000 cases recorded worldwide.[2,3] The first case of BSE confirmed in the United States was identified on Dec. 23, 2003 and was subsequently linked to a Holstein cow that was imported from Canada.[4] Two indigenous cases have since been identified in the United States—one in 2005 and one in 2006. Both of these cases have been classified as BSE by government officials, however in both cases, the infectious prions were slightly higher in molecular weight than those found in European cases and the 2003 BSE case, and have since been described as "atypical."[5]

This report summarizes the proceedings and outcomes of the TSE Think Tank held Nov. 1-3, 2006. Additional information, including presentations and relevant published studies can be accessed at the Beef Industry Food Safety Council's Web site at *www.bifsco.org.*

1 The Economic Impact of BSE on the U.S. Beef Industry: Product Value Losses, Regulatory Costs, and Consumer Reactions, Kansas State University (*http://www.agmanager.info/livestock/marketing/bulletins_2/industry/demand/EconomicImpactofBSEonUSBeefIndustry.pdf*)

2 Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide*(excluding the United Kingdom) (*http://www.oie.int/eng/info/en_esbmonde.htm*)

3 Number of reported cases of bovine spongiform encephalopathy (BSE) reported in the United Kindom (*http://www.oie.int/eng/info/en_esbru.htm*)

4 Timeline of BSE Prevention Measures (*www.bseinfo.org*); United States Department of Agriculture (*www.usda.gov*)

5 Transcript of Tele-News Conference with Agriculture Secretary Mike Johanns and Administrator Ron DeHaven, Animal and Plant Health Inspection Service Regarding BSE Surveillance (*http://www.usda.gov/2006/07/0256.xml*)

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U.S. Department of Agriculture Agricultural Research Service (ARS)

Presentation Summaries

Animal Plant Health Inspection Service (APHIS) Update on U.S. Department of Agriculture (USDA) Surveillance Data

Mark Hall, D.V.M., Ph.D., USDA-APHIS, Veterinary Services National Veterinary Services Laboratory (NVSL), Ames, Iowa

According to Hall, the leading theory is that TSEs are caused by a prion (proteinaceous infectious agent—$PrPS^c$) capable of transforming normal body proteins to an abnormal form. TSEs have a prolonged incubation period and cause a progressive debilitating neurological illness that is ultimately fatal. Pathological changes associated with TSEs are confined to the central nervous system.

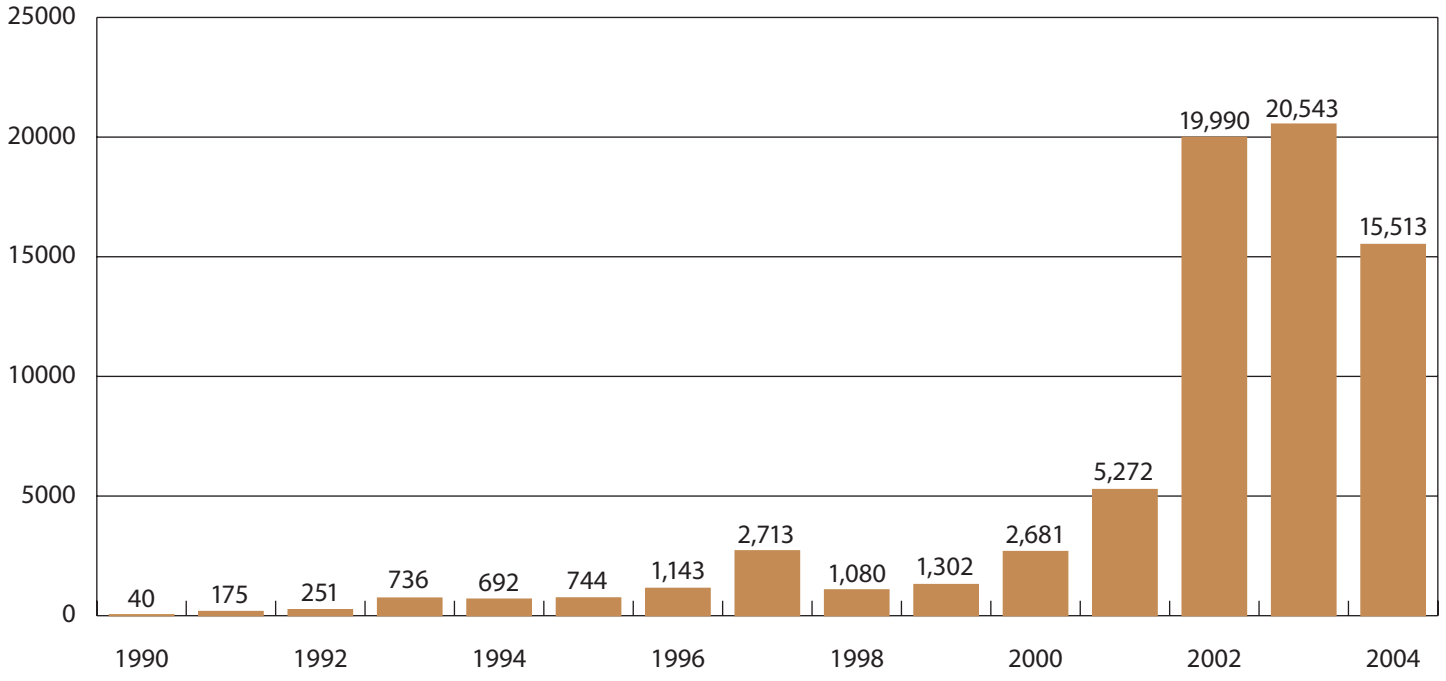
In humans, TSE have manifested themselves in diseases such as Kuru, Gerstmann-Straussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). Creutzfeldt-Jacob Disease (CJD) can occur sporadically (85 to 90 percent of cases—1:1,000,000 incidence rate). Five to 10 percent of cases are traced to familial CJD. Blood transfusions from CJD infected patients has also been a rare source of infection. Variant Creutzfeldt-Jacob Disease (vCJD) is related to the consumption of beef products contaminated with tissue from a BSE-infected bovine. In animals, TSEs are present as Scrapie in sheep, chronic wasting disease (CWD) in deer and elk, transmissible mink encephalopathy (TME) and bovine spongiform encephalopathy in cattle

In cattle, BSE's clinical signs are apparent through changes in behavior, hyperaesthesia (aversion to being touched), weight loss while still exhibiting a healthy appetite, decreases in milk production, ataxia (difficulty standing/lack of motor coordination), difficulty in rising to the point of being considered a "downer," or death. BSE is not a contagious disease; rather, cattle become infected when they consume contaminated feed (meat and bone meal that contains tissue from an infected animal). Clinical signs of the disease do not appear until several years later and affected animals may not show all clinical signs.

Diagnostic Tests

In 1995, the immunohistochemistry (IHC) test was incorporated into surveillance testing in addition to routine histopathology. By 1999, virtually all BSE screening was performed through IHC. In 2001, the National Veterinary Services Laboratory in Ames, Iowa switched to an automated IHC procedure. During 2002 and 2003, the NVSL had tested about 20,000 high-risk animals each year by IHC. Testing procedures at the NVSL evolved from several IHC methods and were validated on European animals that had exhibited clinical signs of BSE.

Figure 1. Number of samples collected through U.S. BSE Surveillance program (May 1990 through April 30, 2004)



During the early part of this decade, experimentation with rapid tests that would be applicable in commercial settings began. To date, there are several tests available, including:

- Prionics Western Blot (Schlieren, Switzerland)
- Biorad Sandwich Immunoassay ELISA (Hercules, Calif.)
- Enfer ELISA (Clonmel, County Tipperary, Ireland)
- Idexx ELISA (Westbrook, Maine)
- Prionics ELISA (Schlieren, Switzerland)

Diagnostic or "rapid" tests are run on fresh or frozen obex brain samples. The sample preparation is generally too complex for field applications. Rapid tests can detect preclinical animals, but they have arguably been associated with false positive results.

BSE surveillance has been ongoing in the United States since the early 1990s. Surveillance efforts have included samples from field cases that have exhibited central nervous system complications (i.e., stumbling, etc.), Veterinary Diagnostic Laboratory data, public health laboratories, carcasses condemned at slaughter due to the exhibition of CNS disorder, and "downers." Hall emphasized that targeted surveillance programs are much more efficient than random sampling. He also stressed that a disease surveillance program should not be considered a food safety test or intervention.

Hall highlighted events related to BSE's emergence in North America. For a timeline of specific BSE events worldwide, see Appendix A. In May of 2003, Canadian animal health officials identified the first case of BSE that was indigenous to North America. Later that year, the first case of BSE to be identified in the United States was found in a dairy cow (Case 1) that had been imported from Canada. The BSE positive was confirmed through an immunohistochemistry test. Beginning in June 2004, USDA began its enhanced BSE surveillance plan, which was scheduled to test at least 268,000 high-risk animals within a 12 to 18 month period.

The first case of BSE identified in the United States (Case 1), was found prior to the implementation of the enhanced surveillance program. Both cases of indigenous BSE in the U.S. (Case 2 and 3) were found after the implementation of the enhanced surveillance program. The cumulative total of animals tested as part of the BSE enhanced surveillance program was 787,711 animals as of Aug. 20, 2006 using the Bio-Rad ELISA. Prevalence estimates for BSE in the U.S. cattle herd are estimated to be at only four to seven animals in the entire adult cattle population (42 million).

In both U.S. indigenous cases (Case 2 and Case 3), the BSE characterization was somewhat unusual. Since that time, improvements in IHC tests allow for better staining of unusual BSE cases. There have also been changes to confirmatory testing procedures that require that the OIE enrichment Western Blot also be performed. All three of the U.S. cases were strongly positive based on the rapid tests. Case 1 had definitive spongiform changes in the obex and the samples exhibited strong immunohistochemical reactions (F99 antibody).

Table 1. Bio-Rad ELISA means for three cases of BSE identified in the United States

Case	Number of Samples	Mean
1	2	1.859
2	6	2.493
3	5	2.403

Table 2. Chronological age of three cows identified as being positive for BSE in the United States

Case	Origin	Age (years)
1	Washington, imported from Canada	6.5
2	Texas	12 (approximate)
3	Alabama	10 (approximate)

Western Blot analyses have also been performed on samples from all three cases. Case 1 contained more PrPSc per brain tissue milligram (mg) equivalent, compared with Cases 2 and 3 (antibody 6H4). Cases 2 and 3 were strongly positive for BSE with antibody P4, while Case 1 was negative or weakly positive with P4. In comparing the three cases further, Hall said that the phenotypes of Case 2 and 3 were unusual, and that they did not exhibit classic BSE histology. Both samples exhibited weaker staining by immunohistochemistry. In Case 2, half of the samples were negative using Western Blot.

Canadian Update

Noel Murray, Ph.D., Canadian Food Inspection Agency, Ottawa, Ontario

Canada's BSE testing program began in 1992 and its objective was to determine if BSE was present by targeting high-risk animals displaying clinical signs consistent with BSE. Over 10,500 animals were tested prior to the detection of BSE in a Canadian-born cow in May 2003. The surveillance program was enhanced in 2004 with new objectives that included gaining a more accurate picture of the level of BSE and to determine the effectiveness of measures designed to manage BSE risks, including the feed ban introduced in 1997.

Canada has identified a minimum annual national target of 30,000 animals be tested. Provincial targets are based on the percentage of the national adult cattle population represented. Since 2004, over 120,000 high-risk animals have been tested, a level that is consistent with OIE guidelines for a BSE-affected country. A federally funded reimbursement program that facilitates the identification and submission of testing candidates augments Canada's surveillance program. Farmers are reimbursed at a rate of $75 (Canadian) per head, veterinarians at $100 per head, and the dead stock/rendering industry at $75 per head. Some provinces provide additional financial reimbursement.

According to Murray, the detection of a few additional cases does not indicate that the prevalence of BSE has increased, but

rather the sensitivity of the surveillance program has increased. More animals are being tested in a targeted surveillance program. "It is reassuring that so few cases have been found amongst over 120,000 high-risk animals tested since 2004," said Murray. The annual incidence of BSE in Canada is 1.3 cases per million (based on the 12-month period from October 2005 to September 2006).

Editor's Note: Since the TSE Summit, Canada has identified a total of 10 cases of BSE. In July 2007, the Canadian government instituted new feed ban controls to further protect against the spread of the disease.[6]

Canada's feed ban is mammalian to ruminant. Prohibited materials include protein that originates from mammals other than pigs or horses. The ban does not include milk, blood, gelatin, rendered animal fat or their products. Salvaged pet food, plate waste and poultry litter may contain prohibited material, but are not approved for feeding to ruminants. Murray said that the awareness and education efforts that have targeted all points along the animal feed chain have been an important aspect of the feed ban. All feed containing prohibited material must have a warning to not feed to cattle, sheep, deer or other ruminants. Canada has instituted detailed, on-site inspections targeting the top end of the feed production chain, including renderers and feed mills.

"It is easy to focus on rare events and possibilities," said Murray during his presentation. "Compliance with the feed ban may not be absolute and rendering may not entirely eliminate infectivity. As a result, some animals may be infected after the feed ban. To better understand a complex and dynamic system, an epidemiological model was developed by CFIA to investigate the impact of the feed ban, which enables us to put rare events and possibilities into perspective."

The model operates under the assumption that a BSE case entered the feed chain in 1990 and that Canada was exposed to an extremely small BSE challenge. Imports of meat and bone meal or feeds containing meat and bone meal were negligible in contrast to what many European countries had experienced. The most likely source of the contamination in Canada was one or a few infected cattle that were imported from the United Kingdom in the 1980s. In Canada, supplementary feeding with meat and bone meal was not extensively practiced as a ready supply of alternative competitively priced protein supplements such as soybean meal and fish meal were widely available. The feed ban achieved a 75 percent reduction in exposure in the first year and rose to 95 percent by 2002. This reduction rate is consistent with the early years following the introduction of the 1988 U.K. ruminant-to-ruminant feed ban.

In analyzing the impact of the feed ban, Murray pointed out that Canada's ban was introduced prior to the detection of BSE cases, which is in stark contrast to the feed bans introduced in the U.K. and several European countries. The levels of the BSE-agent cycling within the cattle populations of these countries had already built up to significant levels. Canadian officials expected the number of BSE cases to peak approximately five years after the introduction of the feed ban—2003. They also expected that there will be a small number of further BSE cases from animals born and infected prior to the feed ban. "The number remaining in the cattle population today would be very small, and many would have already been culled, slaughtered or died," said Murray.

There may be even a few more cases of BSE in animals born after the feed ban, according to Murray, but they do not indicate that the feed ban is failing. Experiences in the United Kingdom and Europe and modeling results indicate that they are epidemiologically unimportant. The potential numbers would be much less than the number of BSE cases from animals infected prior to the feed ban, as the feed ban would have eliminated the vast majority of BSE infectivity. Any cases of BSE identified in animals born after the feed ban may prolong the time required to eradicate BSE, but they are not likely to initiate a new outbreak. The feed ban ensures that the chance of recently infected animals contaminating the feed supply and subsequently infecting more animals is remote.

In June 2006, CFIA announced that SRMs would be banned from all animal feeds, pet foods and fertilizers effective July 2007. The phase-in period of approximately one year is being provided to allow regulated parties to make adjustments to infrastructure and practices. This enhancement will significantly accelerate Canada's progress toward eradicating BSE as the potential for cross contamination that could occur during feed production, transportation, storage, or the misuse of pet food or fertilizers will be virtually eliminated. CFIA will control the collection, treatment, disposal, destruction or alternative use of SRMs through permits. In the short term, SRMs are expected to be managed by way of disposal (e.g. burial in landfills or destroyed by incineration). Alternative uses for SRM, such as processes that can generate biofuel continue to be explored.

Like the U.S., the Canadian Food Inspection Agency has been addressing TSE research initiatives. One of the priorities is to develop tests to support the feed ban by detecting and identifying ruminant products that might be present in animal feed. Canada has also been researching new alternatives for the disposal of SRMs, such as composting. Researchers have been conducting product safety research addressing central nervous system contamination of meat and assaying for PrP in animals susceptible to chronic wasting disease. Work is also being conducted to differentiate TSE strains, including discriminating Canadian isolates of CWD and scrapie from BSE in cattle. Transmission studies to determine the extent to which animals are infected with CWD by the oral route in red deer, reindeer, sheep and goats are being performed. Scientists are also examining the fate of prions in the environment, as well as live animal tests.

The provincial government of Alberta established the Alberta Prion Research Institute (www.prioninstitute.ca) in February 2005 to support researchers working on solutions to the serious scientific and socioeconomic challenges associated with prions.

6 May 2, 2007. Canadian Food Inspection Agency (http://www.inspection.gc.ca/english/corpaffr/newcom/2007/20070502e.shtml)

Typical and Atypical Cases of Bovine Spongiform Encephalopathy

Juergen Richt, Ph.D., U.S. Department of Agriculture, Agricultural Research Service

Richt described the role of prion proteins in prion diseases. Based on research studies, normal PrP (PrP^{c}) is required for infection and disease. Mutations in PrP^{c} can strongly influence susceptibility to TSE disease, or even be the basis for it. Abnormal prion proteins ($PrPS^{c/d/res}$) are associated with neurotoxic events in the central nervous system and are always associated with an infectious agent.

Prion proteins have two conformations. The normal protein is mainly α-helicoidal, sensitive to proteolysis and soluble. The abnormal protein has the same sequence, but has a different shape (i.e., conformational isomer). This protein is toxic and infectious, rich in β-sheet formations, relatively resistant to proteolysis and is insoluble. Multiple alterations occur in the brain as a result of prion diseases, including spongiform degeneration, PrP^{Sc} deposition, astrogliosis and neuronal apoptosis.

Table 3. Animal prion diseases and the year they were first identified.

Disease	Year
Scrapie in sheep and goats	1730
Transmissible mink encephalopathy	1965
Chronic wasting disease in mule deer, elk and moose	1967
Bovine spongiform encephalopathy (BSE or mad cow	1986

The first case of BSE was described in the United Kingdom in 1986. To date, there have been more than 190,000 cases worldwide. A disease of adult cattle, the mean age of onset of BSE is five years. The incubation time after oral infection is typically three years, but can be up to eight years depending on dose. In cattle, transmission occurs via the ingestion of contaminated feed (meat and bone meal). Only a small amount of BSE material is needed for oral transmission (≤ 1 mg of BSE-infected brain material). BSE is not horizontally transmitted.

Until approximately two years ago, researchers assumed that only one strain of BSE existed ("typical" BSE). Recently, "atypical" forms of BSE have been reported in France, Italy, Japan, Belgium and Sweden. Experimental transmission studies that infected cattle with scrapie and chronic wasting disease materials revealed that different TSEs could exist.

According to Richt, the two types of atypical BSE are distinct, and have different proportions of PrPSc glycoforms. Type H (higher molecular weight) was first reported in France and Type L (lower molecular weight) was first reported in Italy. Richt posed the question as to whether atypical BSE is infectious, and said that to date, experimental transmissions into transgenic and nontransgenic mice, cattle and nonhuman primates have been successful. Transmission was also faster than with typical BSE.

In summary, two out of the three cases of BSE identified in the United States show an atypical Western Blot profile (H-type). The H-type BSE is detectable through the U.S. surveillance system, however Richt said that it was important to address whether current surveillance systems for BSE adequately detect atypical strains. To date, the epidemiological investigation for both U.S. born cases (Case 2 and Case 3) did not demonstrate a link to a contaminated feed source, as was the situation in Case 1.

Possible hypotheses about the origin of atypical BSE in cattle include 1) a change in agent, 2) route of infection, 3) scrapie or CWD contamination, 4) sporadic occurrence or 4) a genetic basis or mutation. To determine if a mutation is the origin of atypical BSE cases, a single nucleotide polymorph (SNP) test needs to be developed. During discussion after Richt's presentation, one participant wanted direction on how the potential infectivity and transmissibility of atypical BSE should be communicated to the public. Richt felt that more research is needed to develop any definitive communications beyond what has already been shared, however researchers do believe that SRM removal is adequate for consumer protection.

Editor's Note: Since the TSE Summit, the Spongiform Encephalopathy Advisory Committee (SEAC), which serves to provide independent expert scientific advice on spongiform encephalopathies, such as BSE, CJD and scrapie, has suggested that "atypical BSE" is more correctly described as "unusal or novel BSE," and "typical BSE" is more correctly described as "classical BSE."7

Genetic Variation throughout the Prion Gene in U.S. Cattle Genomes

Michael Clawson, Ph.D., U.S. Department of Agriculture, Agricultural Research Service, U.S. Meat Animal Research Center

Variation in the prion gene (PRNP) correlates with TSE progression in humans, sheep and mice. In cattle a 23-bp insertion/deletion (indel) polymorphism in the putative promoter region and 12-bp insertion deletion within intron I have been associated with German BSE-affected animals. According to Clawson, these polymorphisms are present in U.S. cattle, however most of PRNP has not been characterized in a population as diverse as U.S. cattle. As a result, the extent of PRNP polymorphisms, linkage between PRNP alleles, recombination events and haplotype diversity within PRNP is not known.

Clawson has conducted extensive research to characterize the genetic variation of the prion gene in U.S. cattle. One of his most recent published studies8 characterized the extent of linkage disequilibrium and haplotype networks within PRNP ranging from the promoter past the 3'UTR (25.2 kb) in 192 animals representing 16 beef and five dairy breeds. That research identified 388 total polymorphisms, of which 287 had not been previously reported. The polymorphism alleles define

7 Spongiform Encephalopathy Advisory Committee (*http://www.seac.gov.uk/publicats/publicats.htm*)

8 Clawson, M.L., M.P. Heaton, J.W. Keele, T.P.L. Smith, G.P. Harhay and W.W. Laegreid. 2006. BMC Genetics. 7:51 (*http://www.biomedcentral.com/1471-2156/7/51*).

PRNP by regions of high and low linkage disequilibrium. The research demonstrated that the number of polymorphisms in the prion gene region of U.S. cattle is nearly four times greater than previously described. According to Clawson, these polymorphisms define PRNP haplotypes that may influence BSE susceptibility in cattle.

Chronic Wasting Disease Update

Katherine O'Rourke, U.S. Department of Agriculture, Agricultural Research Service

Chronic wasting disease is a prion disease or transmissible spongiform encephalopathy present in deer, elk and moose. The disease is fatal and can be attributed to the accumulation of PrPd in the brain and lymph nodes. PrPd is a misfolded form of the normal protein PrPc. Chronic wasting disease is a family of disorders that has a prolonged incubation period—often two years. Species that are susceptible to chronic wasting disease under natural conditions include mule deer, Rocky Mountain elk, white tailed deer and Shira's moose. Experimental transmission to some other ruminant species and small carnivores has been achieved.

Researchers have been diagnosing chronic wasting disease through IHC and ELISA. The best tissues for postmortem diagnosis in deer are the lymphoid tissues, but there are very few deer with "brain only PrPd." In elk, researchers can diagnose using both brain and lymphoid tissues. Fifteen percent of positive elk were "brain only PrPd." Live animal testing methods are currently being investigated. Researchers are using a tonsil biopsy or rectal mucosal tissue biopsy. Both are highly specific, however the sensitivity is not yet known, but a study is in progress.

Researchers have observed some genetic differences in the nature of chronic wasting disease among the various wildlife species it affects. For example, in mule deer there are two changes in the prion protein. One change appears to reduce the prevalence and delay the disease. In white tailed deer, there are at least five changes in the prion proteins and one change seems to reduce the prevalence. In elk, there is one change in the prion protein that seems to reduce prevalence and delay disease onset.

As part of the U.S. eradication efforts for chronic wasting disease, 3,000 elk have been euthanized. Fewer than 100 of those animals were positive for the disease. Researchers have asked whether the negative cases were due to a lack of exposure or protective genetics. Additional studies are in progress to determine the answers to those questions. Researchers are also trying to determine how CWD spreads within a population and into new wildlife populations.

In some settings, CWD has been found to be highly transmissible. A study that involved white deer in confinement had 50 to 80 percent with preclinical CWD, as well as fawns under one year with advanced disease. Mule deer in confinement have been observed with 100 percent mortality, while wild mule deer had a 15 percent mortality rate. Farmed elk in depopulated herds at a rate of less than 15 percent, while wild elk were infected at a rate of approximately 1 to 3 percent.

Table 4. Characteristics of TSE in deer and elk versus sheep.

Characteristic	Deer and Elk	Sheep
Transmission Efficiency Deer Elk	 High Moderate	High or moderate, strain dependent
Prions in blood	Yes	Yes
PrPd in lymph nodes of digestive/ respiratory tract	Yes	Yes
Prions in saliva	Yes	Not known
PrPd in placenta	No	Yes
Social behavior, aerosolization, diet and supplements, environmental factors	Unknown	Unknown

Researchers are also examining social behavior and its effect on CWD transmission. Scientists are in the process of developing DNA markers for cervid kinship to better understand if deer are more likely to have CWD if a parent or sibling is positive.

According to O'Rourke, researchers are also addressing whether CWD transmission can occur outside the original endemic area and determining whether wild cervids are transmitting CWD to farmed or domestic animals, and whether the reverse situation is occurring. Animal movements are also being analyzed as wild cervids move further than originally thought. Farmed or captive cervid movement can also play a role in transmission. The transportation of carcasses from hunting is also being examined and is being regulated in some states. Markers are being developed to identify animals or animal parts that come from endemic areas.

The environment may play a role in CWD and researchers are trying to determine if prions persist in the environment. A published study from Iceland found that prions could be maintained in the soil for 16 years, but the results were not conclusive. Researchers are considering whether some environments increase the risk of CWD. There are several studies in press, but the methods have not been standardized and some of the results may be contradictory.

Discovery and Application of a Keritinase that Degrades Prion and Prion-like Proteins

Jason Shih, Ph.D., North Carolina State University

Shih is researching an enzyme that breaks down keratin, which is a key component of chicken feathers. Keratin is an extremely difficult protein to break down, but Shih has isolated an enzyme that degrades it. Potential applications of this keratinase in agriculture include the conversion of feathers into feed protein, feeding the enzyme to livestock to improve protein digestibility, using it as a feed additive for an alternative source of feed protein, as well as the potential destruction of prion proteins.

As part of his research, Shih is also testing keritinase for its ability to break down prion proteins. Based on his work, he is fairly certain that that is the case, but it is unclear as to whether the prion proteins may still retain their infectivity.

Breakout Group Reports

One of the primary goals of the TSE Think Tank was to identify future research and outreach initiatives. Participants broke into smaller discussion groups to focus on specific topics. The outcomes of those discussions are reported below.

Surveillance and Testing/Atypical Cases and Possible Causes of Nonprion TSE

Knowledge gaps and research priorities identified by this joint breakout group:

- Examine non-prion TSES and determine if they should be a research priority.
- Identify a model for communicating to consumers how low the risk to human health is as it relates to atypical BSE cases.
- Determine if the viral theory for transmission has any validity.
- Sporadic CJD cases should be examined with more emphasis on the various subtypes.
- Are there differences in molecular typing that can be related to differences in disease phenotype. Use Kuru as a model in examining transmission routes; once the oral transmission route was eliminated the prevalence disappeared.
- Examine the incubation period of TSEs in United Kingdom cases and determine if carriers exist.
- Investigate the influence of bovine genotype, and whether the occurrence of atypical cases is affected by the genetic makeup of the affected animal. Bos indicus populations should potentially be examined more closely.
- Determine if molecular differences in prion strains make some more susceptible to rendering or heat treatments than others. To support this line of research, more work may have to be done in tissue cultures to differentiate strains.
- Examine if atypical cases are transmitted differently than typical BSE cases. This line of investigation may determine that atypical cases should not be defined as typical BSE cases.
- Do atypical cases affect disease presentation, clinical signs, incubation, transmissibility or diagnostics?
- More closely examine how such a rare disease in cattle can cause a similarly rare disease in humans.
- Determine if atypical BSE is sporadic and naturally occurring in the aged cow population, and if so, should there be more testing of aged, healthy cows?
- Determine if atypical BSE should even be considered a spongiform disease?
- Confirm that specified risk material removal is adequate protection from atypical disease, and that the appropriate SRMS are being targeted for removal.
- Determine incidence of prions in peripheral nerves and/or muscle tissue.
- Examine existing Animal Plant Health Inspection Service data to gain a better understanding of how animals are leaving the population, demographics of culling, age of tested animals, and the geographic distribution of tested animals.

Intervention and Control of TSE

This breakout group identified the following as knowledge gaps that need to be addressed and designated them as research priorities for the future:

- Examine pathogenesis of typical and atypical BSE.
  - SRM identification for atypical BSE cases
  - Determine if and how interventions are impacted by atypical BSE strains
- Investigate the infectivity of bone marrow and cartilaginous tips of spinous processes.
- Determine the economic impacts of potential feed ban changes as proposed by the FDA.
- Research effective disposal of SRMs, dead stock or downers, and slaughter equipment sanitation.
- Examine accurate age determination techniques.
- Continue research to develop live animal tests.
- Conduct a CWD transmission risk assessment.
- Further investigate CNS tissue detection methods.
- Develop protein detection methods for animal feed

Conclusion

After the breakout groups concluded their reports, TSE Think Tank participants came together to prioritize research topics in order of importance.

1. Conduct pathogenesis studies for atypical and typical BSE that would define SRMs for atypical BSE, confirm SRMs for typical, the effects of rendering on atypical BSE, as well as disease presentation characterisitics(symptoms, incubation, morphology).
2. Reseearch the disposal of SRMs, deads/downers, and explore new avenues for equipment sanitation.
3. Research the infectivity of bone marrow and cartilaginous tips of spinous processes.
4. Conduct genetic research of typical and atypical BSE.
5. Develop a methodology for CNS tissue detection, including dorsal root ganglia (DRG).
6. Develop refined diagnostics for atypical BSE.
7. Develop a live animal test for both typical and atypical BSE.
8. Research the potential of contact cohort transmission (natural case).
9. Develop an *in vitro* assay for strain characterization.
10. Increase testing of aged animals.

The participants of the TSE Think Tank felt that even though BSE has ceased being front-page news, it still deserves attention from the research community. Participants felt that the "take away" message from this meeting should center on the following:

1. Establishing an agenda for industry and the scientific community to fill knowledge gaps.
2. The need to better understand atypical BSE has been amplified.
3. Continued research funding will be critical to understanding BSE and its ramifications.

"This meeting was an opportunity to assess completed and current research on this issue," said Mike Engler, a feedlot operator in Amarillo, Texas and chairman of the Joint Industry Beef Safety Committee. "There's a definite need for more research on TSE diseases and the think tank provided a forum for U.S. and international experts to discuss where our resources should be focused."

Appendix A: Timeline of BSE-related Events

Timeline of BSE Prevention Measures	
November 1986	BSE is first diagnosed in the U.K.
July 18, 1988	Ruminant meat-and-bone meal (MBM) is banned from inclusion into cattle feed in the U.K.
July 21, 1989	USDA/APHIS bans the importation of ruminant animals (cattle, sheep, goats, deer, elk and buffalo) from countries with confirmed cases of BSE.
November 1989	USDA/APHIS enacts emergency ban on the importation of high risk ruminant products (including meat-and-bone-meal) from countries with confirmed cases of BSE. Formal regulation to follow.
1990	FDA intensifies microbiological review of new drug applications for human drug products derived from bovine sources. USDA initiates a surveillance program and begins testing for BSE in cattle showing signs of possible neurological disease.
December 6, 1991	USDA/APHIS enacts formal regulation to restrict the importation of ruminant meat and edible products, and bans high risk by-products of ruminant origin from countries known to have BSE.
1993	USDA/APHIS expands BSE surveillance program to include examination of brain tissue from non-ambulatory or “downer” cows.
January 1993	BSE epidemic in U.K. peaks with 1,000 new cases reported per week.
1994	USDA/APHIS implements immunohistochemistry testingmethod for BSE.
March 20, 1996	British government announces possible link between BSE and 10 cases of a new human TSE called new variant Creutzfeldt-Jakob Disease (nvCJD).
March 29, 1996	National livestock organizations and professional animal health organizations in the U.S. announce a voluntary program to discontinue the use of ruminant-derived protein in ruminant feed. The FDA and USDA announce their intentions to determine if additional regulations are necessary to prevent the introduction and or amplification of the BSE agent in the United States.
January 1997	FDA proposes a ban on the use of ruminant products in livestock feed.
June 2, 1997	FDA issues a regulation banning the use of high risk mammalian protein in animal feed. Limited exceptions include blood, milk or gelatin products, and equine and porcine protein, which are derived from species not known to develop TSEs naturally.
August 4, 1997	FDA rule that banned the use of high risk mammal-derived protein by-products in bulk feeds for cattle becomes effective.
October 3, 1997	FDA rule that banned the use of high risk mammal-derived protein by-products in bagged feed for cattle becomes effective.
December 12, 1997	USDA/APHIS bans imports of all live ruminants and high risk ruminant products from Europe.
April 24, 1998	USDA/APHIS enters into a cooperative agreement with HarvardUniversity’s School of Public Health to analyze and evaluate the USDA’s BSE prevention measures.
March 2000	Due to concerns about foot-and-mouth disease, U.S. restricts imports of live ruminants and animals from Japan. Restrictions continue as the first case of BSE found outside of Europe is reportedin Japan, in September 2001.The ban from March 2000 remains in effect.
December 7, 2000	APHIS prohibits all imports of rendered animal protein products from Europe, regardless of species.
January 29, 2001	The National Cattlemen’s Beef Association hosts meeting with the FDA Center for Veterinary Medicine, USDA Animal and Plant Health Inspection Service, the feed industry and meat packing and rendering industries to discuss ensuring full compliance with FDA rulings.
February 3, 2001	Officials from the NCBA, Canadian Cattlemen’s Association and Conferacion Nacional Ganadera of Mexico sign a joint statement pledging to keep BSE out of North America.
April 2001	U.S. beef industry implements an affidavit system by which cattle producers provide written confirmation that cattle posted for sale have not been fed prohibited materials per the 1997 feed ban regulations. The U.S. feed industry develops a certification program, which assures that certified feed suppliers comply with FDA feed ban regulations.
Fall 2001	USDA announces enhancements to its cattle surveillance system, including dividing the country into eight regions to assure testing accounts for regional differences yet assures uniform national surveillance.With this regional structure, USDA will double the number of cattle tested for BSE in 2002 as compared to the previous year.
November 2001	HarvardCenter for Risk Analysis releases its BSE risk assessment study commissioned by the federal government. The report finds the risk of BSE ever occurring in the U.S. is very low. *http://www.aphis.usda.gov/lpa/issues/bse/bse-riskassmt.html*

Timeline of BSE Prevention Measures	
February 2002	The beef industry develops an affidavit form for feed suppliers to certify that the feeds sold to cattle producers contain no prohibited materials.
FY 2000	U.S. surveillance is increased to testing 19,990 cattle brains.
May 20 2003	Canada confirms first indigenous case of BSEin a single 6-year old Alberta beef cow. The U.S. closes the border to live cattle and beef imports. The Canadian government conducts an exhaustive investigation that turns up no additional cases (Canadian Food Inspection Agency *www.inspection.gc.ca*) The probe concludes that the most likely explanation for infectivity was that, prior to the feed ban, the cow consumed livestock feed containing contaminated rendered animal proteins.An international team of BSE experts, called to Canada to review the official investigation, confirmed the findings. Acting on recommendations from the international team, Canada tightens prevention to include new rules on specified risk materials (SRM). For U.S. reaction to the Canada case, go to *http://www.usda.gov/news/releases/2003/05/bg0166.htm*
August 2003	USDA announces it will allow certain Canadian ruminant products to enter the U.S. under permit. These include boneless beef from cattle under 30 months of age and boneless veal from calves under the age of 37 weeks.
September 2003	FDA reports that the compliance rate on the ruminant feed ban is near 100% for feed mills. *http://www.fda.gov/cvm/index/updates/BSEInspec03.htm*.
October 2003	USDA announces a proposed rule to amend its BSE regulations to allow the importation of certain low-risk live ruminants and ruminant products from minimal BSE risk regions under specified conditions. The proposed rule places Canada on a list of countries considered minimal risk for BSE. USDA also releases the results of the second Harvard BSE risk assessment. The study found that even if infected animals or ruminant feed material entered the U.S. from Canada, the risk of it spreading within the U.S. herd is low; that any possible spread would now have been reversed by the controls put in place in the late '90s; and that the disease would be eventually eliminated from the U.S over a period of time. For more information go to *http://www.aphis.usda.gov/lpa/issues/bse/bse.html*
December 23, 2003	USDA announces a single, "presumptive positive" case of BSE in Washington state in a 6 ½-year-old dairy cow.The cow originated in Canada.The diagnosis was made through histopathology and immunohistochemical testing at the National Veterinary Services Laboratory, Ames, Iowa.USDA/APHIS launches an exhaustive investigation that ultimately involves more than 75,000 animals on 189 premises. USDA/FSIS initiates a beef recall.
December 23, 2003-December 31, 2003	Fifty-three countries ban imports of US beef and beef products.
December 25, 2003	The OIE International Reference Laboratory in Weybridge, England confirms the BSE diagnosis.
December 30, 2003	USDA/FSIS announces new rules banning all "downer" cattle from the human food chain, removing certain animals and specified risk material (SRM) and tissues from the human food chain, requiring additional process controls for establishments using advanced meat recovery (AMR), holding meat from cattle that have been targeted for BSE surveillance testing until the test has confirmed negative and prohibiting air-injection stunning of cattle.(See *http://www.fsis.usda.gov/oa/news/2004/bseregs.htm* for more information.)
January 12, 2004	Interim final rules announced by USDA on December 30 become effective.
January 26, 2004	FDA announces new rules to strengthen existing BSE firewalls.New measures include banning a wide range of bovine material from human food, dietary supplements and cosmetics, and strengthening the 1997 feed ban through an extended list of banned feeding and manufacturing practices.See *http://www.hhs.gov/news/press/2004pres/20040126.html* for more information.
February 4, 2004	International panel releases report on measures relating to the incidence of Bovine Spongiform Encephalopathy (BSE) in the U.S.
February 9, 2004	USDA completes BSE field investigation.See *http://www.aphis.usda.gov/lpa/issues/bse/updates/bse_update02-09-04.htm* for a complete summary.
June 1, 2004	Following an international scientific review panel's recommendation (see the report at *http://www.aphis.usda.gov/lpa/issues/bse/bse_sec_adv_comm.doc*) USDA begins an enhanced BSE surveillance program targeting cattle from highest-risk populations, as well as a random sampling of animals from the aged cattle population. See the initial program announcement at: *http://www.usda.gov/Newsroom/0105.04.html* See the latest details on the enhanced surveillance program at *http://www.aphis.usda.gov/lpa/issues/bse/bse.html*
June 24, 2005	USDA announces the first indigenous case of BSE found in the United States, traceback activities indicate the animal was approximately 12 years old, and born and raised on a ranch in Texas. Updates on the epidemiological investigation can be found at *http://www.aphis.usda.gov/lpa/issues/bse/bse.html*
March 15, 2006	On March 15, NVSL completed immunohistochemical testing on tissues from a native-born cow, confirming the second native case of BSE in the United States. *http://www.aphis.usda.gov/newsroom/hot_issues/bse/downloads/EPI_Final5-2-06.pdf*

Timeline of BSE Prevention Measures	
July 20, 2006	USDA announces new BSE surveillance program. *http://www.usda.gov/wps/portal/!ut/p/_s.7_0_A/7_0_1OB?contentidonly=true&contentid=2006/07/0255.xml*
July 27, 2006	Harvard BSE Risk Assessment Update released. *http://www.fsis.usda.gov/Science/Risk_Assessments/index.asp*
Jan. 4, 2007	USDA proposes to allow additional imports from BSE Minimal Risk countries. *http://www.usda.gov/wps/portal/!ut/p/_s.7_0_A/7_0_1OB?contentidonly=true&contentid=2007/01/0001.xml*
Feb. 5, 2007	Japan confirms its 32nd case of BSE. *http://news.yahoo.com/s/afp/20070205/hl_afp/healthjapanmadcowus_070205091552*
Feb. 7, 2007	Canada confirms its ninth case of BSE. *http://www.inspection.gc.ca/english/anima/heasan/disemala/bseesb/ab2007/9notavie.shtml*
May 2, 2007	Canada confirms its 10th case of BSE. *http://www.inspection.gc.ca/english/corpaffr/newcom/2007/20070502e.shtml*
May 9, 2007	Japan tests fail to show BSE infection from young cattle. *http://www.alertnet.org/thenews/newsdesk/T267692.htm*
May 22, 2007	OIE classifies the United States as a controlled risk country for BSE. *http://www.usda.gov/wps/portal/!ut/p/_s.7_0_A/7_0_1OB?contentidonly=true&contentid=2007/05/0149.xml*
July 12, 2007	FSIS publishes final rule prohibiting processing of “downer” cattle. *http://www.fsis.usda.gov/News_&_Events/NR_071207_01/index.asp*

For more information contact:

National Cattlemen's Beef Association

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