

## BOVINE SPONGIFORM ENCEPHALOPATHY

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### Definition [top](#)

Bovine spongiform encephalopathy (BSE), widely known as "mad cow disease," is a chronic, afebrile, degenerative disease affecting the central nervous system (CNS) of cattle.

Bovine spongiform encephalopathy belongs to the family of diseases known as the transmissible spongiform encephalopathies (TSE's). These diseases are caused by a transmissible agent that is yet to be fully characterized. They share the following common characteristics:

- a. A prolonged incubation period of months or years;

- b. A progressive debilitating neurological illness that is always fatal;
- c. When examined by electron microscopy, detergent-treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapie-associated fibrils (SAF's);
- d. Pathological changes appear to be confined to the CNS and include vacuolation and astrocytosis;
- e. The transmissible agent elicits no detectable specific immune response in the host.

Specific types of TSE's include scrapie, which affects sheep and goats; transmissible mink encephalopathy; feline spongiform encephalopathy; chronic wasting disease of deer and elk; and five rare diseases in humans: kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia (FFI), and new variant Creutzfeldt-Jakob disease (nvCJD).

### Etiology [top](#)

The clinical, pathological, and molecular genetic features of BSE, as well as other transmissible spongiform encephalopathies, have led to speculation on the nature of the etiologic agent and the pathogenic mechanisms of the disease. There are three main theories on the nature of the scrapie agent:

1. The virus theory, in which the virus would have to have unusual biochemical and biophysical characteristics that would help explain the remarkable physicochemical properties (12, 24, 39, 40).
2. The prion theory, in which the agent is conceived of being composed exclusively of a host-coded normal cellular protein (PrP<sup>C</sup>) that becomes partially protease resistant (PrP<sup>BSE</sup>) — most likely through a post-translational conformation change after infection. In this theory there is no nonhost component of the agent. That is, a specific informational molecule (nucleic acid e.g., RNA or DNA) is not present (5, 36).
3. The virino theory, which states that the agent consists of a host-derived protein coat, (PrP being one of the candidates for this protective protein) and a small noncoding regulatory nucleic acid (14, 21).

All of the proposed theories have some degree of validity. Proponents of the virus

and virino theories have concluded that the existence of different scrapie strains unequivocally proves the presence of a nucleic acid component of the infectious agent which, as in conventional viruses, may undergo mutations responsible for phenotypic variations. The problem with these theories is that no agent-specific nucleic acid has been convincingly identified to copurify with infectivity (15, 25, 28, 32, 42). Moreover, chemical, enzymatic, or physical treatments that usually inactivate or degrade nucleic acids have no effect on the transmissible properties of the infectious agent (3, 4, 27, 31). Possible reasons for this are that the amount of nucleic acid of the putative agent is too small to be detected with available techniques and that its tight bond to the protein protects it from chemical or physical inactivation. Also weakening the virus and virino theories is the inability to identify any virus particles under the electron microscope (6, 10), and the failure of an infected host to generate an immune response. Recently small particles resembling virus structures have been observed by electron microscopy (33).

The prion model involves propagation of a protein-only agent ( $\text{PrP}^{\text{BSE}}$ ) whereby  $\text{PrP}^{\text{C}}$  can assume various tertiary structures caused by a combination of host genetics and the introduction of altered (infectious) PrP ( $\text{PrP}^{\text{BSE}}$ ). More simply stated, the structure of the infecting  $\text{PrP}^{\text{BSE}}$  imprints upon the normal cellular precursor ( $\text{PrP}^{\text{C}}$ ) and results in a conformation change to the protease-resistant form. It is suspected that "strain" differences result from mutations in the PrP gene that may cause proteins "flip" and change shape. Several explanations for scrapie strain genetics in the context of the prion theory have been suggested but none have been proven (35, 41, 46).

It should be pointed out that the prion theory fails to explain a) how the PrP of the infecting agent originally assumed the aberrant structure associated with infectivity, and b) how the different structures originated as a function of the different strains. Although numerous scrapie strains can be differentiated in a single host (i.e., sheep), the PrP agents associated with these strains have not shown any biochemical and molecular differences; thus, BSE seems to be caused by a single strain type. This BSE strain is different from historical or contemporary isolates from sheep or goats with natural scrapie, as determined by study of incubation periods and brain "lesion profiles" in mice.

Regardless of whether the prion ( $\text{PrP}^{\text{BSE}}$ ) is or is not the etiologic agent, the partially protease-resistant form of the prion protein is a marker of infection.

### Host Range [top](#)

Bovine spongiform encephalopathy has been experimentally transmitted to the

following species via intracerebral (IC) inoculation: cattle, sheep, and goats (17), mink (38), pigs (13), marmosets (1), macaques (22), and mice (16). Intracerebral transmission was attempted in hamsters but was not successful. Via the oral route, BSE has been successfully transmitted to cattle, sheep, and goats (17); mice (2); and mink (38). Oral transmission has not been successful in swine. Parenteral and oral transmission has also been attempted in chickens with no evidence of disease thus far.

A transmissible spongiform encephalopathy has been diagnosed in eight species of captive wild ruminants as well as exotic (cheetahs, pumas, a tiger, and an ocelot) and domestic cats. There have been about 81 domestic cat cases of feline spongiform encephalopathy (FSE) in Great Britain and in 1 domestic cat each in Norway, Northern Ireland, and Liechtenstein. The agent isolated from several of these cases using strain typing in mice is indistinguishable from BSE in cattle, which suggests that FSE is actually BSE in exotic and domestic cats. This also appears to be true for the other ruminants. Epidemiological evidence suggests BSE-contaminated feed to be the primary source of infection in these species (30).

Other cases of spongiform encephalopathy have been reported in kudu, eland, nyala, gemsbok, and a few exotic cats. These too are thought to be linked to contaminated feed.

It has also been suggested that 23 cases (as of January 31, 1998) of a variant form of CJD (nvCJD) (a human disease) in Great Britain (U.K. Department of Health, March 2, 1998) and 1 case in France may be linked to exposure to BSE before the introduction of a specified bovine offal (SBO) ban at slaughter in 1989. The SBO ban excludes from human consumption brain, spinal cord, and other tissues with potential BSE infectivity.

### **Geographic Distribution [top](#)**

Worldwide there have been more than 170,000 cases since the disease was first diagnosed in 1986 in Great Britain. Over 95 percent of these cases have occurred in the United Kingdom. The disease has also been confirmed in native-born cattle in Belgium, France, Ireland, Luxembourg, the Netherlands, Northern Ireland, Portugal, and Switzerland but is not known to exist in the United States.

### **Transmission [top](#)**

Different scientific hypotheses have been advanced concerning the origins of BSE. The epidemiologic data suggest that BSE in Great Britain is an extended common source epidemic involving feed containing TSE-contaminated meat and bone meal

as a protein source. The causative agent is suspected to be from either scrapie-affected sheep or cattle with a previously unidentified TSE.

Changes in rendering operations in the early 1980's — particularly the removal of a solvent-extraction process that included a steam-heat treatment — may have played a part in the appearance of the disease and the subsequent amplification of the agent in the food chain. A ban on feeding animal protein of ruminant origin to ruminants was enacted in Great Britain in July 1988(50).

In Great Britain the epidemic peaked in 1992-93, when approximately 1,000 cases were being reported per week. In 1998 it remains on the decline with approximately 100 cases reported per week. Cases that have been detected in other countries appear to be a result of importations of live cattle or, more significantly, contaminated feed from Great Britain.

There is no evidence that BSE spreads horizontally; that is, by contact between unrelated adult cattle or from cattle to other species.

New evidence suggests that maternal transmission may occur at an extremely low level. Results of British research show low levels of transmission of BSE from affected cows to their offspring. These results demonstrated that there is approximately a 9 percent increase in the occurrence of BSE in offspring of BSE-affected dams as compared with calves born to dams where BSE was not detected. The study did not ascertain if this was the result of genetic factors or true transmission. The research did, however, point out that, at this level, if maternal transmission does occur, it alone will not sustain the epidemic (51).

In the naturally infected animals, the agent has been identified by mouse bioassay in the brain, spinal cord, and retina. The route of inoculation into the mice was intracranial. The naturally infected animals were adult cattle exhibiting clinical signs of disease (16).

Mice fed milk, mammary gland, placenta, lymph nodes, or spleen have failed to develop the disease or to establish subclinical infection of the lymphoreticular system within their natural lifespan (29).

Another study was conducted to examine the pathogenesis of BSE in cattle; that is the replication (tissue distribution) of the agent during the incubation period. This study, which has not yet been completed, has identified the agent via mouse bioassay in the distal ileum of the experimentally infected calves. It is thought that the agent may be associated with the lymphoid tissue of the intestines. The calves were 4 months of age at the time of oral dosing. First isolation of the agent in the

distal ileum was made at 6 months after oral dosing. Subsequent isolations from the distal ileum were made at 10, 14, and 18 months after dosing (47). Recently this study has also identified infectivity in bone marrow, trigeminal ganglion, dorsal root ganglion, brain, and spinal cord (48).

No infectivity has been found by parenteral or oral challenge, or both, in over 40 other tissues from clinically ill cattle using the mouse bioassay. It appears as if the distribution of the BSE agent is not as diverse as the scrapie agent in sheep. However, there is a possibility that the agent is present but is at such low levels that the bioassay is not sensitive enough to detect it (30).

### **Incubation Period** [top](#)

The incubation period usually ranges from 2 to 8 years. Following the onset of clinical signs, the animal's condition gradually deteriorates until the animal becomes recumbent, dies, or is destroyed. This usually takes from 2 weeks to 6 months. Most cases in Great Britain have occurred in dairy cows (Friesians) between 3 and 6 years of age (50). The youngest confirmed case occurred in a 20-month-old heifer, and the oldest case was found in a cow 18 years of age.

### **Clinical Signs** [top](#)

Cattle affected by BSE develop a progressive degeneration of the nervous system. Affected animals may display changes in temperament, abnormalities of posture and movement, and changes in sensation. More specifically, the signs include apprehension, nervousness or aggression, incoordination, especially hind-limb ataxia, tremor, difficulty in rising, and hyperaesthesia to sound and touch. In addition, many animals have decreased milk production or loss of body condition, or both, despite continued appetite.

### **Gross Lesions** [top](#)

There is no gross lesion associated with BSE.

### **Morbidity and Mortality** [top](#)

In Great Britain, 19 percent of the dairy herds and 1.6 percent of the beef herds have had one or more cases of BSE. This difference is believed to result from the fact that dairy calves were fed a higher level of protein supplement. The average incidence in herds in Great Britain has been 1.75 cases. However, there have been a few herds with over 30 cases. Affected animals die.

## Diagnosis [top](#)

### Field Diagnosis [top](#)

A field diagnosis of BSE is based on the occurrence of clinical signs of the disease. A bovine animal that has signs of a CNS disturbance should be observed over time (at least 2 weeks) to determine whether the signs become progressively more severe. If, after this interval, improvement or recovery has not taken place, BSE should be suspected and the animal humanely euthanized.

### Specimens for the Laboratory [top](#)

Because the BSE agent is considered a human pathogen, protective clothing, gloves, and face protection should be worn when performing the necropsy. The entire brain should be removed intact with a portion of the cranial cervical spinal cord attached. Portions should be placed in a plastic bag and submitted unfixed. The remainder of the brain should be fixed in 10 percent buffered formalin solution. One cerebral hemisphere is removed by cutting the brain stem through the space between the cerebellum and cerebrum with a longitudinal cut between the cerebral hemispheres.

### Laboratory Diagnosis [top](#)

Bovine spongiform encephalopathy currently must be confirmed by histopathological examination of brain tissue. Bilaterally symmetrical degenerative changes are usually seen in the gray matter of the brain stem. These changes are characterized by vacuolation or microcavitation of nerve cells in the brain stem nuclei. The neural perikarya and axons of certain brain stem nuclei contain intracytoplasmic vacuoles of various sizes, that give the impression of a spongy brain. Hypertrophy of astrocytes often accompanies the vacuolation (49). A diagnosis may also be made by the detection of SAF's using electron microscopy.

Two supplemental tests are available to enhance the diagnostic capabilities for BSE. These are immunohistochemistry and the Western blot technique. In the past, if the brain tissue was not harvested shortly after the animal's death, autolysis often made it very difficult to confirm a diagnosis by histopathology. These tests allow for the possibility of confirming a diagnosis of BSE by detecting PrP<sup>BSE</sup> even if the brain has been frozen or autolyzed .

### Differential Diagnosis [top](#)

Differentials for BSE include rabies, listeriosis, nervous ketosis, milk fever, grass tetany, lead poisoning, and other toxicities or etiological agents affecting the nervous or musculoskeletal system of adult cattle.

### **Treatment** [top](#)

There is no known treatment for BSE or any of the TSE's.

### **Vaccination** [top](#)

There is no preventative vaccine.

### **Control and Eradication** [top](#)

Bovine spongiform encephalopathy from foreign sources may be prevented by the implementation of import regulations prohibiting live ruminants and ruminant products (especially meat, bone meal, and offal) from countries where BSE may exist. Because the origin of BSE remains unknown, preventing an epidemic of BSE would involve, at a minimum, the prohibition of feeding ruminant proteins to ruminants. The prevention program of any country should also include an active surveillance effort focused on high-risk cattle for the early detection of BSE. Most countries of the world have prohibited the importation of cattle and bovine products from countries known to have BSE. In addition many countries have taken steps to enact regulations prohibiting the feeding of ruminant proteins to ruminants. This is true even in countries such as Australia and New Zealand with no known animal TSE's.

Agricultural officials in countries known to have BSE have taken a series of actions to control and, it is to be hoped, eradicate BSE. These include making BSE a notifiable disease, prohibiting the inclusion of certain animal proteins in ruminants' rations (the feed bans vary depending on the amount of BSE detected), and depopulating certain populations of cattle thought to be of higher risk because of epidemiological findings.

To prevent human exposure to the BSE agent numerous countries have established prohibitions on the inclusion of high risk material in foods, pharmaceuticals, cosmetics, and so forth.

### **U.S. Actions** [top](#)

With an active surveillance program in place for 8 years, BSE has not been

detected in the United States. The United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and industry groups are actively working to maintain this status. The measures USDA, Animal and Plant Health Inspection Service (APHIS), has taken in this regard include prohibitions or restrictions, or both, on certain animal and product imports, ongoing surveillance for the disease in the United States, preparation of an emergency response plan in the unlikely event an introduction were to occur, and continuing educational efforts. The Animal and Plant Health Inspection Service actively shares information and coordinates closely with other Federal agencies, as well as the States, livestock and affiliated industries, veterinary and research communities, and consumer groups, to ensure that the United States has a uniform approach to transmissible spongiform encephalopathies based on sound scientific information.

A comprehensive surveillance program has been implemented by APHIS in the United States to ensure timely detection and swift response in the unlikely event that an introduction of BSE were to occur. This surveillance program entails the location of imports from countries known to have BSE and targeted active and passive surveillance for either BSE or any other TSE in cattle.

To locate each of the 496 British cattle that were imported into this country between January 1, 1981, and July 1989, APHIS has conducted a traceback effort. In July 1989, the United States prohibited the importation of ruminants from countries affected with BSE. As of March 1998, only 17 of these animals are known to be alive in the United States, and these are being carefully monitored by APHIS personnel on an ongoing basis. In addition, five head of cattle imported from Belgium in 1996 are now under quarantine. In cooperation with the states and industry, APHIS continues to purchase these animals for diagnostic purposes. No evidence of BSE has been found in any of these imported animals.

The United States has had an aggressive, active surveillance program for BSE since May 1990. Bovine spongiform encephalopathy is a notifiable disease, and there are more than 250 Federal and State regulatory veterinarians specially trained to diagnose foreign animal diseases, including BSE. The Animal and Plant Health Inspection Service leads an interagency surveillance program, which includes the Food Safety Inspection Service (FSIS) and the Centers for Disease Control (CDC). The surveillance samples include field cases of cattle exhibiting signs of neurological disease, cattle condemned at slaughter for neurological reasons, rabies-negative cattle submitted to public health laboratories, neurological cases submitted to veterinary diagnostic laboratories and teaching hospitals, and random sampling of cattle that are nonambulatory at slaughter. As of February 21, 1998, over 6,600 brains had been examined for BSE or another form of a transmissible spongiform encephalopathy in cattle. No evidence of either condition has been detected by histopathology or immunohistochemistry.

As of December 12, 1997, APHIS has prohibited the importation of live ruminants and most ruminant products from all of Europe until a thorough assessment of the risks can be made. The new restrictions apply to Albania, Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Federal Republic of Yugoslavia, Finland, Germany, Greece, Hungary, Italy, Former Yugoslav Republic of Macedonia, Norway, Poland, Romania, Slovak Republic, Slovenia, Spain, and Sweden.

This action was taken because, in the past year, the Netherlands, Belgium, and Luxembourg have reported their first cases of BSE in native-born cattle. There is evidence that European countries may have had high BSE risk factors for several years and less than adequate surveillance. Additionally, Belgium reported that the cow diagnosed with BSE was processed into the animal food chain.

The Food and Drug Administration (FDA) has recently established regulations that prohibit the feeding of most mammalian proteins to ruminants. The effective date of this regulation was August 4, 1997.

### **Public Health [top](#)**

#### **BSE and CJD — Human Health Concerns [top](#)**

On March 20, 1996, the U.K.'s Spongiform Encephalopathy Advisory Committee (SEAC) announced the identification of 10 cases of a new variant form of CJD (nvCJD). All of the patients developed onset of illness in 1994 or 1995. The following features describe how these 10 cases differed from the sporadic form of CJD:

- The affected individuals were much younger than the sporadic CJD patient. Typically, sporadic CJD patients are over 63 years old. The average patient age for the variant form of CJD is 27.5 (range of 16 to 42) years.
- The course of the disease in the nvCJD averaged 13 months. Sporadic CJD cases average a 6-month duration.
- In the variant cases, electroencephalographic (EEG) electrical activity was not typical of sporadic CJD.
- Although brain pathology was recognizable as CJD, the pattern was different from normal CJD, and evidenced large aggregates of prion

protein plaques.

Epidemiologic and case studies have not revealed a common risk factor among the cases of nvCJD. According to the SEAC, all victims were reported to have eaten beef or beef products in the last 10 years, but none had knowingly eaten brain material. One of the affected individuals had been a vegetarian since 1991 (52).

The SEAC concluded that, although there was no direct scientific evidence of a link between BSE and nvCJD, on the basis of current data and in the absence of any credible alternative, the most likely explanation was that the cases were linked to exposure to BSE before the introduction of control measures; namely, the specified bovine offal (SBO) ban in 1989.

Research reported in later 1996 and 1997 has presented further evidence to support a causal association between nvCJD and BSE. Two significant studies published in the October 2, 1997, edition of *Nature* led the SEAC to conclude that the BSE agent is very likely to be the cause of nvCJD. Dr. Moira Bruce and colleagues at the Institute for Animal Health in Edinburgh, Scotland, inoculated three panels of inbred mice and one panel of crossbred mice with BSE, nvCJD, and sporadic CJD. Interim results indicate that mice inoculated with BSE show the same pattern of incubation time, clinical signs, and brain lesions as mice inoculated with tissues from patients with nvCJD. This provides evidence that BSE and nvCJD have the same signature or are the same "strain." In addition classical CJD and known scrapie strains were not similar to nvCJD or BSE (9).

Results from another study published by Dr. John Collinge and colleagues of Imperial College School of Medicine, London, United Kingdom, strongly support Bruce's results. Collinge's paper reports experimental transmission of BSE to transgenic mice expressing only human PrP (20).

The Health and Safety Executive in the United Kingdom now advises that BSE must be considered a biological agent (human pathogen) within the meaning of the Control of Substances Hazardous to Health Regulations 1994 (45).

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