

Bovine Spongiform Encephalopathy

*Mad Cow Disease,
BSE*

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the Center for
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Importance

Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease, caused by a prion, that mainly affects cattle. Other ruminant species, cats, non-human primates and humans are occasionally affected; this disease is called feline spongiform encephalopathy (FSE) in cats, and variant Creutzfeldt–Jakob disease (vCJD) in people. BSE is a relatively new disease that was first reported in the United Kingdom in the 1980s. It is spread by ingestion; animals or humans become infected when they eat prion-containing tissues from an infected animal. Cooking and standard disinfection procedures do not destroy this agent. Infected animals or people do not become ill for years; however, the disease is always progressive and fatal once the symptoms develop.

The origins of BSE are unknown; however, the recycling of ruminant proteins in ruminant feed amplified this prion and caused an explosive epidemic in the U.K in the 1980s and 1990s. This epidemic peaked in 1992, with almost 1,000 new cases diagnosed each week. Although cases continue to be detected, control measures have greatly decreased their prevalence; fewer than 15 bovine cases were reported annually in the U.K. during 2009-2011. BSE also spread to many European countries, North America, parts of Asia, and possibly other areas of the world. The presence of BSE in a country can result in trade sanctions, as well as increased public concern about meat safety. Many nations, including the U.S., conduct control and surveillance programs. Many countries have also passed new regulations to prevent BSE-containing tissues from entering human or animal food supplies.

As a result of increased surveillance, BSE prions that differ from the prion causing ‘classical’ BSE have been identified at very low levels in cattle populations. Currently, it is thought that these “atypical” prions may represent a spontaneous form of prion disease. Some experiments suggest that an atypical prion might have given rise to the BSE epizootic when it was amplified in cattle feed.

Etiology

BSE is a member of the transmissible spongiform encephalopathies (TSEs), a group of neurodegenerative disorders caused by unconventional disease agents. These agents are resistant to the treatments that ordinarily destroy bacteria, spores, viruses, and fungi. They are generally thought to be prions, although a minority opinion suggests that TSEs may be caused by viruses or retroviruses. Prions are infectious proteins that appear to replicate by converting a normal cellular protein into copies of the prion. The cellular protein, which is called PrP^c, is found on the surface of neurons. Pathogenic isoforms of PrP^c are designated PrP^{res}; PrP^{Sc} or PrP^{TSE} are other names for this protein. Prions that cause different diseases (e.g. BSE or scrapie) are considered to be different strains of PrP^{res}.

In addition to the ‘classical’ BSE prion, at least two atypical BSE prions can be found in cattle. One has higher molecular mass fragments than classical BSE and is called ‘H-type’ BSE or H-BSE; the other has a lower molecular mass and is called ‘L-type’ BSE or L-BSE. Some authors call the disease caused by the latter organism ‘bovine amyloidotic spongiform encephalopathy (BASE).’ Atypical BSE prions are thought to represent additional strains of BSE. Currently, the most likely hypothesis is that these prions arise spontaneously in cattle, similarly to some prion diseases in other species (e.g., spontaneous Creutzfeldt–Jakob disease in humans). Atypical L-BSE has been reported to change to a classical BSE phenotype on transmission to inbred mice or to some transgenic mice. Similarly, H-BSE developed features of classical BSE in some wild type mice. This has led to the suggestion that one of these prions may have originally given rise to the BSE epidemic after amplification through the food chain.

Geographic Distribution

Cases of BSE have been reported in indigenous cattle in most European countries, Canada, the U.S., Israel, and Japan. This disease was seen in imported cattle in the Falkland Islands and Oman. Some countries including Iceland, Australia, and New Zealand appear to be free of BSE. The presence or absence of this disease cannot be determined in countries without adequate surveillance programs.

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Atypical BSE prions have been reported in Europe, the U.S., Canada, and Japan, as the result of surveillance programs for BSE. They are also likely to exist in other countries.

Transmission

BSE is usually transmitted when an animal or human ingests tissues containing the BSE prion. Young animals may be particularly susceptible to infection; some studies suggest that most cattle become infected with BSE during the first six months of life. The prions are thought to replicate initially in the Peyer's patches of the ileum, then are transported via the peripheral nerves to the central nervous system (CNS). In cattle, prions can accumulate in the brain as early as 24 months after infection. The risks of transmission from various tissues are still incompletely understood; however, the highest prion concentration occurs in the CNS and ileum. In naturally infected cattle, BSE prions have been found mainly in the brain, spinal cord, retina, and distal ileum, but more sensitive techniques have recently detected this agent in the dorsal root ganglia, peripheral nerves (including the optic, facial and sciatic nerves), and adrenal glands. In experimentally infected cattle, it has been reported from the CNS, dorsal root ganglia, trigeminal ganglion, thoracic ganglia, some peripheral nerves, distal ileum (particularly in the Peyer's patches), jejunum, ileocecal junction, cecum, colon, myenteric plexus of the intestines, adrenal glands, tonsils, and bone marrow. In one animal, immunostaining detected prions in the macrophages of the subiliac lymph nodes but not other lymph nodes tested. In this animal, very weak immunostaining was also detected in the renal tubular epithelial cells, the thymus, and the islets of Langerhans. Using a very sensitive system (transgenic mouse bioassay), infectivity was recently detected in the tongue and nasal mucosa of cattle in the terminal stages of the disease, although no prions could be found by immunoblotting or the protein misfolding cyclic amplification (PMCA) method. Unpublished data suggested that BSE prions might occur in the lymphoid tissues of nictitating membranes, but no evidence to confirm this has been published.

In some tissues, the quantity of prions may be low or the incidence rare, and the risk of transmission is uncertain. Some tissues may contain prions only in the late stages of the disease. For example, the accumulation of these agents in the peripheral nerves and adrenal gland seems to coincide with or follow prion accumulation in the CNS. Classical BSE has not been found in muscle, except in one sample tested by mouse bioassay, where infectivity was thought to be associated with the endings of the sciatic nerve. However, meat could become contaminated with CNS tissues during slaughter or processing. For this reason, high-risk slaughter and processing techniques have been banned in many nations (see 'Prevention'). Epidemiological evidence and transmission studies suggest that BSE is not transmitted in milk, semen, or embryos.

There is no evidence that BSE is transmitted horizontally between cattle; however, there is an unexplained increase in the risk of BSE among the offspring of infected animals. In one study, the risk that a calf would develop BSE appeared to be higher when the dam was in the later stages of infection (i.e., nearer to the onset of clinical signs). These observations have led to speculation that vertical transmission might be possible. If it occurs, vertical transmission seems to be rare, and the route is unknown. One model suggested that the cumulative risk of BSE transmission from dam to offspring is about 2%; however, the confidence interval included zero.

BSE transmission in experimentally infected sheep resembles transmission in cattle, but the prions are more widely disseminated in the body, and additional routes of transmission may occur. In sheep inoculated orally, BSE prions are readily found in many lymphoid tissues including the spleen, lymph nodes, and gut-associated lymphoid tissue (GALT), as well as in the CNS. Blood-borne transmission has been demonstrated in this species. Transmission from two ewes to their lambs occurred in an experimental flock; it is not known whether this event took place *in utero* or soon after birth.

Atypical BSE

In cattle, some studies report that the tissue distribution of atypical L-BSE and H-BSE seems to resemble that of classical BSE, with prions detected mainly in the CNS. (There are, however, some differences in the pattern of distribution within the brain.) H-BSE and L-BSE have also been found in peripheral nerves and sensory receptors (muscle spindles) and the trigeminal ganglion in some studies, and L-BSE was detected in the adrenal gland. In a recently published study, PrP^{res} was reported to occur in the muscles of L-BSE infected cattle by immunostaining, and infectivity was found in muscle homogenates using a transgenic mouse bioassay.

Very little is known about the potential for vertical transmission. One calf born to a cow in the late stages of infection with L-BSE did not have any evidence of infection.

Transmission to humans and iatrogenic spread

In humans, variant Creutzfeldt-Jakob disease usually results from the ingestion of BSE prions. Based on studies in humanized transgenic mice, some authors have suggested that BSE isolates from sheep and goats might be more readily transmitted to humans than isolates from cattle. Iatrogenic transmission has also been seen. Probable person-to-person spread was reported in several patients who received blood transfusions from asymptotically infected individuals. There is also potential for transmission by routes such as transplantation or the use of prion-contaminated equipment during surgeries. Prions can be found in the brain, spinal cord, dorsal root ganglia, trigeminal ganglia, retina, optic nerves, and lymphoid tissues of humans with vCJD. Although prions are particularly common in the spleen, tonsils, appendix and

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other gut-associated lymphoid tissues (GALT), they can also be found in lymph nodes throughout the body. Prions have been found in the appendix as early as two years before the onset of clinical disease. They have not been demonstrated in human blood, but this may be due to the insensitivity of the assays used to detect these agents. Person-to-person transmission of vCJD does not occur during casual contact.

Origins of the BSE epidemic

The origins of BSE are not well understood. This disease was first reported in the 1980s, but it was probably present in cattle since the 1970s or earlier. The two most popular hypotheses are that BSE originated as a spontaneous PrP^c mutation in cattle, or that it came from a mutated scrapie prion that contaminated ruminant feed. Other sources suggest that BSE might have originated from a wildlife population or a human TSE agent. Once the BSE agent entered cattle populations, it was amplified by recycling tissues from infected cattle into ruminant feed supplements, mainly as meat-and-bone meal (MBM). MBM is a rendered concentrate derived from animal offal and carcasses. Rendering cannot completely inactivate prions, but the epidemic may have been facilitated by changes in rendering practices that allowed more prions to survive.

Banning ruminant tissues from ruminant feed has significantly reduced the number of new cases of BSE, but cases have been reported in cattle born after these regulations came into effect (“born-after-the-ban” cases). These cases might be caused by the use of imported feed components produced under inadequate quality controls, illegal feeding of ruminant proteins, or cross-contamination of cattle feed with swine or poultry feed. Theoretical possibilities include inadequate heating of bone meal or tallow used in concentrates and milk replacers, horizontal transmission, or environmental reservoirs. Current diagnostic techniques are not sensitive enough to detect very low levels of prions, and there is little information on prion survival in the environment; however, hamster-adapted scrapie prions have been shown to survive in the soil for at least three years.

Disinfection

Prions can differ in their resistance to inactivation, and one study found that BSE prions from cattle were more resistant than prions from human spontaneous CJD, mouse-passaged BSE prions, or hamster prions.

Decontamination of prion-contaminated tissues, surfaces, and environments is difficult. These agents are highly resistant to most disinfectants (including formalin), heat, ultraviolet radiation, and ionizing radiation, particularly when they are protected in organic material or preserved with aldehyde fixatives, or when the prion titer is high. Prions can bind tightly to some surfaces, including stainless steel and plastic, without losing infectivity. Prions bound to metal seem to be highly resistant to decontamination. Few effective

decontamination techniques have been published. A 1-2 N sodium hydroxide solution, or a sodium hypochlorite solution containing 2% available chlorine, has traditionally been recommended for equipment and surfaces. Surfaces should be treated for more than 1 hour at 20°C (68°F). Overnight disinfection is recommended for equipment. Cleaning before disinfection removes organic material that may protect prions. In experiments, milder treatments including a phenolic disinfectant, an alkaline cleaner (KOH with detergents), and an enzymatic cleaner combined with vaporized hydrogen peroxide were shown to inactivate scrapie prions. The alkaline cleaner and phenolic disinfectant were also effective against BSE and vCJD prions. New commercial decontaminants have been developed for prions. In one experiment, the most effective commercial reagents, using a rodent prion on stainless steel wires, were those that contained proteolytic agents. One of these solutions was prepared in an 2 M NaOH alkali carrier, which may have contributed to its effectiveness. A commercial alkali/detergent reagent was unable to completely decontaminate the wires.

Physical inactivation of prions can be carried out by porous load autoclaving at 134-138°C (273-280°F) for 18 minutes at 30 lb/in², but residual infectivity has been demonstrated in some studies. Autoclaving items in water is more effective than autoclaving without immersion. Dry heat is less effective; hamster-adapted scrapie prions can survive dry heat at temperatures as high as 360°C (680°F) for an hour. A combination of chemical and physical decontamination can be more effective than either procedure alone; chemical disinfection should be carried out first, then the items should be rinsed and autoclaved. Anecdotal evidence suggests that decontamination of contaminated facilities is very difficult.

Even the harshest combination of chemical and physical disinfection is not guaranteed to destroy all prions. In experiments, a stainless-steel wire remained infectious after cleaning with sodium hydroxide and autoclaving. Surgical instruments that have undergone repeated cycles of cleaning and disinfection have transmitted the sporadic (genetic) form of CJD iatrogenically. For this reason, disposable equipment and instruments may be recommended instead of disinfection during some medical procedures.

Infections in Humans

Incubation Period

The incubation period for vCJD is difficult to establish with certainty; however, the average incubation period is estimated to be 11 to 12 years, and incubation periods up to 16 years have been reported. In three cases transmitted in blood transfusions, the incubation period was 6 to 8.5 years. For comparison, some other human prion diseases have similar median incubation periods, but have been reported up to 40 years after exposure.

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Clinical Signs

The symptoms of vCJD are broadly similar to the sporadic (genetic) form of CJD, but usually appear in younger patients. The median age of onset is 26 years (range 12 to 74 years). The first signs are usually psychiatric symptoms, such as anxiety, depression, insomnia, social withdrawal, and/or persistent painful sensory symptoms. In most patients, frank neurological signs such as gait disturbances, ataxia, incoordination, memory loss, slurring of speech, and tremor appear a few months later; however, neurological signs coincide with or precede psychiatric symptoms in a minority of patients. Cognitive function gradually deteriorates. Chorea, dystonia, myoclonus, visual disturbances, and dementia typically develop late in the course of disease. Most patients die in six months to two years.

Communicability

Person-to-person transmission of vCJD does not occur during casual contact. Probable human-to-human spread has been reported in several patients who received blood transfusions from asymptotically infected individuals. Other iatrogenic routes of transmission may be possible, including transmission in transplants or by contaminated equipment during surgeries. In humans, prions can be found in the CNS and many lymphoid tissues including the tonsils. Prions have been found in the appendix as early as two years before the onset of clinical disease.

Diagnostic Tests

A tentative diagnosis can be made before death by the history, clinical signs, and cortical atrophy on magnetic resonance imaging (MRI) of the brain. The electroencephalogram (EEG) is sometimes normal during the early stages of disease, but later develops characteristic abnormalities. A definitive diagnosis can be made if the abnormal prion protein is found in tonsil biopsies by immunoblot (Western blot) or immunohistochemistry. In other cases, the diagnosis is made by microscopic examination of brain tissue, usually at necropsy. Numerous amyloid plaques surrounded by vacuoles are found in vCJD; such plaques are seen in only 5-10% of cases of sporadic (genetic) CJD. Large amounts of prion protein can be found around the plaques by immunohistochemistry.

Treatment

No treatment is available, other than supportive care.

Prevention

Variant Creutzfeldt-Jakob disease can usually be avoided by not eating tissues from BSE-infected cattle. Some nations conduct active surveillance of cattle at slaughter (using rapid tests) to detect cases of BSE. Active surveillance for BSE has been conducted in the E.U. since 2001; however, the age limits have increased since the programs began. As of 2011, most E.U. member states must

test cattle over the age of 48 months that die, undergo emergency slaughter, are killed for reasons other than human consumption, or display certain abnormalities at antemortem inspection. Healthy cattle over the age of 72 months and intended for human consumption must also be tested. Lower age limits apply to cattle from a few E.U. member states and some other areas. Japan conducts unusually rigorous testing. At one time, the Japanese government required all slaughtered cattle to be tested for BSE. Since 2005, it has required only cattle that are 21 months of age or older to be tested; however, there has been public resistance to relaxing the testing requirements, and local authorities have continued to test all slaughtered cattle regardless of age. Some countries with a low incidence of disease, including the U.S., test only a percentage of cattle at slaughter. In the U.S., surveillance is targeted particularly at high risk cattle such as nonambulatory animals and those with neurological disease. These animals cannot be used in human food, and the carcass is held until testing is complete. Because BSE has been detected in goats, and sheep are also susceptible to experimental infection, some countries may also conduct BSE surveillance in small ruminants.

Tissues that have a high risk of transmitting BSE have been banned from human food in many countries. In the U.S., prohibited tissues include the brain, skull, eyes, trigeminal ganglia, dorsal root ganglia, spinal cord, and most of the vertebrae from cattle 30 months of age and older. The tonsils and distal ileum from all cattle are also banned. In the E.U., banned tissues include the skull (including the brain and eyes but not the mandible) and spinal cord in cattle over 12 months of age, and the spinal column in cattle over 30 months of age. The tonsils, entire intestines, and mesentery are not allowed from any cattle. Slaughter and processing techniques that have a high risk of contaminating muscle tissues with CNS have been prohibited in many countries, including the U.S.

Person-to-person transmission of vCJD can be reduced by the use of disposable surgical instruments in high risk surgeries, when this disease is suspected. Because prions can be found in the tonsils, some authors also suggest that disposable equipment always be used during tonsillectomies, in countries with a significant risk of this disease. Transmission in blood cannot be completely prevented with current techniques; however, many countries do not allow people who have spent time in the U.K. and/or other European countries to be blood donors. Some countries have taken other measures, such as universal leucodepletion of blood, to reduce the risk of vCJD. Prion filters have been developed to reduce infectivity in plasma, but are still being evaluated and are not in wide use. Some countries import fresh frozen plasma from low-risk countries for patients without dietary exposure to BSE (e.g., patients born after 1996 in the U.K.).

Although laboratory or abattoir-related cases have not been reported, veterinarians and laboratory workers should

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always take precautions when conducting necropsies on BSE suspects or handling tissues; BSL-3 is the recommended level of protection. Standard precautions include the use of protective clothing and the avoidance of penetrating injuries, contamination of abraded skin, and ingestion. A negative pressure laminar flow hood should be used for tissue manipulations whenever possible. Because prions may be able to survive in the environment for years and are difficult to disinfect, precautions should be taken to avoid contamination of surfaces and equipment. Disposable plastic-coated paper sheets can be used to protect tables and other surfaces. Disposable instruments and work clothing can also be used. No vaccine is available.

Morbidity and Mortality

The prevalence of vCJD is unknown. Most cases have been seen in people who lived in either the U.K. or France during the peak of the BSE epidemic. As of April 2012, 176 cases of vCJD had been reported in the U.K. The incidence peaked in 2000, when 28 cases were diagnosed, and gradually fell to five cases per year in 2005. Between 2006 and 2011, two to five cases were reported each year. As of April 2012, 25 cases had been reported from France, as well as four from the Republic of Ireland, five from Spain, three each from the United States and the Netherlands, and two from Canada, Portugal, and Italy. Japan, Saudi Arabia, and Taiwan have each reported one case. To date, all cases of vCJD in the U.S. seem to have been acquired in other countries. The number of people who are infected but asymptomatic is unknown. Based on the pattern of infection in the U.K, some sources suggest that, at most, 70 additional cases can be expected; however, surveillance conducted on appendectomy samples in the U.K. suggested a prevalence of 237 cases per million population, with 95% confidence intervals of 49–692. Another study, based on samples from tonsils, estimated a prevalence of 1 case per 10,000 population.

Variant Creutzfeldt–Jakob disease is usually seen in young patients. The reason is unknown, but it is possible that children and adolescents are more susceptible to infection than adults. The median age of onset is 26 years for vCJD (range 12 to 74 years); in contrast, it is 65 years (range 15 to 94 years) in the sporadic (genetic) form of Creutzfeldt–Jakob disease. People who are homozygous for methionine at codon 129 in the PrP^C protein have an increased risk of developing vCJD. All clinical cases have occurred in people with this genotype. One infection was reported in a person who was heterozygous for methionine/valine at this codon, but did not develop vCJD symptoms. This person became infected in a blood transfusion and died of unrelated causes after five years. It is not known whether people with resistant genotypes (valine/valine or methionine/valine) are completely resistant to the development of disease, or simply have a longer incubation period. Once the symptoms of vCJD develop, this disease is always fatal.

As of 2012, no human infections have been reported with atypical BSE prions, but these prions are also likely to be of zoonotic concern. In particular, L-BSE seems to be more virulent than classical BSE in intracerebrally inoculated macaques and humanized transgenic mice, with a shorter incubation period and more rapid progression. There is currently no evidence that this is the case for H-BSE; however, H-BSE (like L-BSE) is capable of changing to resemble classical BSE in transgenic mice.

Infections in Animals

Species Affected

BSE mainly occurs in cattle, but the host range of this prion is unusually broad compared to most prions. BSE has been reported from exotic ruminants in zoos; affected species include nyala (*Tragelaphus angasi*), kudu (*Tragelaphus strepsiceros*), gemsbok (*Oryx gazella*), eland (*Taurotragus oryx*), Arabian oryx (*Oryx leucoryx*), scimitar-horned oryx (*Oryx dammah*), ankole cattle, and bison (*Bison bison*). Rare field cases have been documented in goats, and experimental infections have been reported in both sheep and goats. European red deer (*Cervus elaphus elaphus*) are susceptible to oral exposure at a high dose, as well as to intracerebral inoculation, and develop neurological signs. BSE prions have also caused disease in various felids including housecats, cheetahs (*Acinonyx jubatus*), pumas (*Felis concolor*), ocelots (*Felis pardalis*), tigers (*Panthera tigris*), and Asian golden cats (*Catopuma temminckii*). (See the feline spongiform encephalopathy factsheet for details on infections in felids.) Two lemurs at a French zoo were apparently infected in contaminated feed. In addition, the BSE agent has been experimentally transmitted to mink, mice, marmosets, squirrel monkeys (*Saimiri sciureus*) and cynomolgus macaques (*Macaca fascicularis*). Pigs could be infected by the intracranial, intravenous, and intraperitoneal routes, but short-term feeding trials did not cause disease. One study reported that sea bream (*Sparus aurata*) might be susceptible to infection, although they did not develop clinical signs.

L-BSE can infect cynomolgus macaques by intracerebral inoculation. It has also been transmitted to lemurs by the oral route, with the development of neurological signs. L-BSE and H-BSE can infect mice by intracerebral inoculation.

Incubation Period

The incubation period for classical BSE is estimated to be 2 to 8 years in cattle. The peak incidence of disease occurs in four to five year old animals. Atypical BSE is usually detected in cattle that are at least eight years of age. Some research suggests that the incubation period might be shorter for atypical L-BSE than classical BSE; however, this is based on comparisons in intracerebrally inoculated cattle and bovinized transgenic mice.

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The incubation period in experimentally infected sheep varies with the animal's age and genetic susceptibility, and the route of exposure and dose. In genetically susceptible sheep, the incubation period was 21 to 38 months for animals inoculated orally at six months of age, and 18 to 24 months in lambs inoculated orally at two weeks of age. In genetically resistant (ARR/ARR) sheep, the incubation period was approximately 3 to 5 years.

One European red deer challenged by the oral route developed clinical signs after approximately 4 years, 9 months, while four other deer were still healthy more than 5 years after challenge. In experimentally infected macaques inoculated orally, the incubation period was 3.6 to 5 years.

Clinical Signs

Cattle with classical BSE

Bovine spongiform encephalopathy is a neurological disease that usually has an insidious onset in cattle. The symptoms may include gait abnormalities (particularly hindlimb ataxia), hyperresponsiveness to stimuli, tremors, and behavioral changes such as aggression, nervousness or apprehension, changes in temperament, and even frenzy. The combination of behavioral changes, hyperactivity to stimuli, and gait abnormalities is highly suggestive of BSE, but some animals exhibit only one category of neurological signs. Pacing, a modified gait in which the legs move in lateral pairs, occurred in 25% of the cattle with BSE in one study, and may be suggestive of this disease. Intense pruritus is not usually seen in cattle, but some animals may lick or rub persistently. Nonspecific signs include loss of condition, weight loss, teeth grinding (possibly due to visceral pain or neurological disease), and decreased milk production. Decreased rumination, bradycardia, and altered heart rhythms have also been reported. The symptoms of BSE usually worsen gradually over a few weeks to six months, but rare cases can develop acutely and progress rapidly. Rapid, acute onset neurological disease seems to be particularly common in exotic ruminants in zoos. Once the symptoms appear, BSE is always progressive and fatal. The final stages are characterized by recumbency, coma, and death.

Cattle with atypical BSE

The features of atypical BSE in cattle are still incompletely understood. Most atypical strains have been found in asymptomatic cattle during routine surveillance, in fallen stock ('downer' cattle), or at emergency slaughter. However, H-BSE associated with neurological signs was reported in a 19-year-old zebu bull (*Bos indicus*) at a zoo. Experiments (all using intracerebrally inoculated cattle) have reported varying clinical signs, with some researchers concluding that L-BSE can be distinguished clinically from classical BSE, and others reporting that the spectrum of clinical signs overlaps to a greater or lesser extent between all forms of BSE.

In Friesian and Alpine brown cattle, one group of researchers reported that an Italian isolate of L-BSE mainly

causes a form characterized by inactivity, mental dullness, and muscle atrophy, which could be distinguished from classical BSE. The early clinical signs included muscle fasciculations, a dull coat, decreased alertness, low carriage of the head, and mild kyphosis. These signs progressed to muscle atrophy, which began in the gluteal region and progressed to involve other areas, with relative sparing of forelimb muscles. Although a "downer" cow was reported in this study, other animals did not develop ataxia or difficulty rising. However, sudden falls were seen. The animals in this study were reported to be hyperresponsive to tactile facial stimuli, but not to light or sound. In this experiment, the same breeds inoculated with classical BSE prions developed behavioral changes including aggressiveness, bellowing and head shaking, as well as postural abnormalities and hyperresponsiveness to stimuli.

Another group reported that, in Holstein-Friesian cattle inoculated with German isolates of H-BSE and L-BSE, the first signs were weight loss and loss of condition. Animals tended to separate from the herd and carried their head low. However, these cattle were hyperresponsive to acoustic and visual stimuli as well as tactile facial stimuli, similarly to cattle with classical BSE. Ataxia and difficulty rising were also reported in this experiment. These researchers concluded that, although the initial signs appear to be more nonspecific and subtle in atypical BSE, the differences are not sufficient to unambiguously distinguish these forms from classical BSE.

Another experiment used Danish Holstein/ Aberdeen Angus crosses inoculated with an Italian L-BSE strain and an H-BSE strain. Both "dull" and "nervous" forms of the illness were reported in this study. Behavioral, sensory, and motor signs were all seen, and cattle infected with H-BSE and L-BSE had similar clinical signs. Low head carriage and separation from the herd did not occur consistently, and most of the animals had no signs of dullness. Instead, many animals became hyperreactive to external stimuli including tactile and facial stimuli. No cattle developed tremors. In this study, the cattle tended to develop dysmetria and have difficulty in rising, early in the course of the disease, but none progressed to permanent recumbency (unlike animals with classical BSE that develop ataxia).

A study that used a Japanese L-BSE isolate in Holstein cattle reported decreased activity, hyperresponsiveness to stimuli, ataxia mainly of the hindlegs, difficulty rising, and little aggression.

Sheep with classical BSE

Various neurological signs have been reported in experimentally infected sheep. In one study, Cheviot sheep mainly developed ataxia with minimal pruritus, and died in a few days to a week. In indigenous French breeds, the symptoms included ataxia and intense pruritus with loss of fleece. These animals deteriorated slowly and died in approximately three months. A third study mainly used ARQ homozygous Suffolk and Romney sheep, but also

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included a few individuals of other breeds, and reported that the clinical signs were similar in all animals. Pruritus was detected in all clinically affected sheep in this study (however, it should be noted that this sign was also reported in 29% of the sheep that did not have evidence of BSE at slaughter). Other signs in some animals included behavioral changes, teeth grinding, movement abnormalities including tremor and ataxia, hyperresponsiveness to auditory stimuli or decreased menace response in a few animals, and weight loss and loss of body condition. Altered behavior combined with ataxia and pruritus was detected in 40% of these sheep. The course of the illness lasted 16 to 20 weeks before animals were culled due to the progression of neurological signs.

Goats with classical BSE

The few BSE cases that have been reported in naturally infected goats were discovered during routine surveillance at slaughter. One goat was reported to be a scrapie suspect. Neurological signs have been reported in experimentally infected animals. In one study, the disease was characterized by ataxia and tremors, and progressed rapidly in intracerebrally inoculated goats; however, the signs in orally inoculated goats were mainly lethargy and weight loss, which progressed to recumbency over three weeks. Ataxia was not seen in orally inoculated goats, and neither intracerebrally nor orally inoculated goats had signs of pruritus. In another study, intracerebrally inoculated Saanen goats developed abnormalities in movement (e.g., ataxia, tremors, postural deficits, and especially hypermetria) and hyperresponsiveness to stimuli. Over the course of the experiment, sniffing and nibbling of the animal handlers and instruments changed to aversive behavior, including head tossing or shaking, or kicking, and these signs became more pronounced with time. One goat carried its head low when undisturbed and was inappetent. Other signs in some animals included pruritus, an absent menace response, teeth grinding, and weight loss.

Post Mortem Lesions [Click to view images](#)

Gross lesions are not found in BSE, with the exception of nonspecific signs, such as emaciation or wasting. The histopathologic lesions are confined to the CNS. Neuronal vacuolation and non-inflammatory spongiform changes in the gray matter are characteristic of the disease in cattle. These lesions are usually but not always bilaterally symmetrical. Amyloid plaques are not typical of classical BSE or infection with H-BSE, but are associated with L-BSE prions. Similar spongiform changes occur in experimentally infected sheep and macaques.

Communicability

There is no evidence that the BSE agent is transmitted horizontally between cattle; however, the offspring of infected animals have an increased risk of developing this disease. If vertical transmission is possible, the route is unknown.

Diagnostic Tests

There is no live animal test for BSE. This disease is usually diagnosed by detecting prions (PrP^{res}) in the CNS. Accumulations of prions can be found in unfixed brain extracts by immunoblotting, and in fixed brains by immunohistochemistry. In addition, several rapid diagnostic tests based on enzyme-linked immunosorbent assays (ELISAs), automated immunoblotting (Western blotting) and lateral flow devices (LFD) are available. Rapid tests allow large numbers of samples to be screened, and are often used in surveillance and slaughter testing. Positive samples in rapid tests are traditionally confirmed with more specific assays such as immunohistochemistry or immunoblotting. However, the OIE now states that confirmation of positive results with a second rapid test is acceptable under some circumstances. Preferred test combinations, which are not in danger of producing false positives by the use of shared reagents, are listed on the OIE website. Two rapid tests can only be used to confirm a BSE case; a negative result on the confirmatory test is not adequate to rule out BSE, and should be investigated with other assays. A diagnosis of BSE may also be confirmed by finding characteristic prion fibrils called scrapie-associated fibrils (SAF) with electron microscopy in brain extracts. Some of these tests can be used on frozen or autolyzed brains. Techniques used diagnostically to detect prions are relatively insensitive compared to assays for other types of pathogens; prions cannot usually be detected in the brain until 3 to 6 months before the onset of disease.

Atypical prions can be detected with the same tests, including rapid tests, in animals infected with H-BSE or L-BSE. A complete evaluation of the rapid tests has not been done for these prions. The distribution patterns of H-BSE and L-BSE in the brain differ somewhat from that of classical BSE, as well as from each other; however, all three prions can be detected in the obex. Atypical prions can be differentiated from classical BSE prions by their biochemical properties, for example by immunoblotting. H-BSE has higher molecular mass fragments than classical BSE. It also reacts with a monoclonal antibody to an N-terminal epitope that is not found in classical BSE after proteinase K cleavage. L-BSE has a lower molecular mass than classical BSE prions. Its glycosylation pattern differs from classical BSE, and it has an unusual deposition pattern characterized by amyloid plaques.

Histological examination of the brain can be very helpful in diagnosis, but some animals in early stages of infection have few or no spongiform changes. In addition, BSE can be detected by transmission studies in mice; however, an incubation period of several months makes this technique impractical for routine diagnosis. Serology is not useful for diagnosis, as antibodies are not made against the BSE agent.

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Treatment

There is no treatment for BSE. Suspect animals are usually euthanized for testing.

Prevention

BSE can be prevented by not feeding ruminant tissues that may contain prions to susceptible species. Complete avoidance is generally necessary, as cooking or rendering cannot completely inactivate prions. Many nations have now banned the use of either ruminant or mammalian proteins, with certain exceptions such as milk and blood, in livestock feed. The specific bans, and protein sources prohibited, vary with the country. In some countries, bans also apply to other animal feeds, or even to fertilizer. The latter measures can help prevent cross-contamination and accidental exposure of cattle to BSE prions. Preventing prions from re-entering the ruminant food chain can interrupt transmission and control BSE epidemics; however, due to the long incubation period, the number of BSE cases may not decline for some time. In addition, countries may place trade bans on the importation of live cattle and certain ruminant proteins from affected countries.

BSE suspects are usually euthanized for testing. These carcasses cannot be used as food and must be destroyed. In the U.K., BSE carcasses are rendered at 133°C (3 bar pressure) for at least 20 minutes. Surveillance can help prevent infected animals from being used in food. Some nations conduct active surveillance of cattle at slaughter (using rapid tests) to detect cases of BSE. Active surveillance for BSE has been conducted in the E.U. since 2001; however, the age limits have increased since the programs began. As of 2011, cattle that must be tested (with rapid tests) in most E.U. member states include animals over the age of 48 months that die, undergo emergency slaughter, are killed for reasons other than human consumption, or display certain abnormalities at ante-mortem inspection. Healthy cattle over the age of 72 months and intended for human consumption must also be tested. Lower age limits apply to cattle from some other areas. Japan has unusually strict requirements. At one time, the Japan government required all cattle to be tested for BSE. Since 2005, only cattle that are 21 months of age or older must be tested; however, there has been public resistance to relaxing testing requirements, and local authorities have continued to test all slaughtered cattle regardless of age. Some countries may also conduct BSE surveillance in small ruminants.

Some countries with a low incidence of disease, including the U.S., test only a percentage of cattle at slaughter. In the U.S., surveillance is targeted particularly at high risk cattle such as nonambulatory animals and those with neurological disease. These animals cannot be used in human food, and the carcass is held until testing is complete. The U.S. also conducts passive surveillance for BSE. When an infected animal is identified, the affected herd is quarantined, and the source of the infection is

investigated. Due to the increased risk of BSE in the offspring of infected cattle, they are usually traced and euthanized.

Tissues that have a high risk of transmitting BSE have been banned from human food in many countries. In the U.S., prohibited tissues include the brain, skull, eyes, trigeminal ganglia, dorsal root ganglia, spinal cord, and most of the vertebrae from cattle 30 months of age and older. The tonsils and distal ileum from all cattle are also banned. In the E.U., banned tissues include the skull (including the brain and eyes but not the mandible) and spinal cord in cattle over 12 months of age, and the spinal column in cattle over 30 months of age. The tonsils, entire intestine, and mesentery are not allowed from any cattle. Slaughter and processing techniques that have a high risk of contaminating muscle tissues with CNS have been prohibited in many countries, including the U.S.

Morbidity and Mortality

Classical BSE is seen most often in four to five year old cattle, particularly dairy animals. This disease is always fatal once the symptoms appear. The prevalence of BSE varies widely. At one time, the estimated prevalence in various countries ranged from more than 100 cases per million cattle to fewer than two cases per million. The latter are defined as World Organization for Animal Health (OIE) 'minimal risk' countries for BSE. Control measures have greatly decreased the prevalence in the most severely affected nations since that time.

BSE epidemics were reported in several European countries. The first outbreak occurred in the U.K., where more than 180,000 cases have been confirmed since the 1980s. The U.K. epidemic peaked in 1992, with nearly 1,000 new cases confirmed each week. At the time, the annual incidence in affected herds was approximately 2-3%. As a result of control measures (particularly feed bans), the incidence declined to approximately 5-10 new cases per week in 2004. This number continued to drop, with the annual incidence decreasing to 99 confirmed cases in 2006, 35 cases in 2008, and 7 to 11 cases each year between 2009 and 2011. The peak of the epidemic curve occurred later in countries where feed bans were established more recently. In the U.S., only four cases of BSE have been reported. One case occurred in an animal imported from Canada. Three additional cases have been reported in indigenous cattle; one was caused by the H-form of atypical BSE. The most recent case (April 2012) was infected by an L-BSE prion, but the specific form has not yet been specified.

As of 2012, approximately 60 cases of L-BSE or H-BSE have been identified worldwide as a result of surveillance for classical BSE. The incidence of atypical BSE appears to be much lower than classical BSE. Its prevalence among cattle in France and Germany may be as low as 1 case per 3 million adult cattle. Nearly all L-BSE and H-BSE prions have been detected in cattle over the age

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of 8 years, with the exception of an L-BSE prion reported from a 23-month old steer in Japan.

Cases of BSE are very rarely reported in goats. Infections have not been seen in sheep or deer other than experimentally infected animals. Surveillance conducted in Europe suggests that the prevalence of BSE is very low in sheep, if it occurs at all. Estimates of the maximum proportion of sheep TSE cases that could be BSE range from 0.7% to 5%. Experimentally infected sheep that are genetically resistant to scrapie seem to have some resistance to BSE, but are not immune to infection or disease.

Internet Resources

- Canadian Food Inspection Agency
<http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/bse/eng/1323991831668/1323991912972>
- Centers for Disease Control and Prevention
<http://www.cdc.gov/ncidod/dvrd/bse/index.htm>
- European Commission. TSE/BSE
http://ec.europa.eu/food/food/biosafety/tse_bse/index_en.htm
- The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bi/mvm_bkindex.htm
- The National Creutzfeldt-Jakob Disease Surveillance Unit, United Kingdom.
<http://www.cjd.ed.ac.uk/index.htm>
- United Kingdom. Department for Environment Food and Rural Affairs. Bovine Spongiform Encephalopathy
<http://animalhealth.defra.gov.uk/managing-disease/notifiable-disease/bse.html>
- United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service. Bovine Spongiform Encephalopathy
http://www.aphis.usda.gov/newsroom/hot_issues/bse/index.shtml
- USDA Foreign Agricultural Service. Bovine Spongiform Encephalopathy
<http://www.fas.usda.gov/DLP/BSE/bse.html>
- United States Food and Drug Administration. Bovine Spongiform Encephalopathy
<http://www.fda.gov/animalveterinary/guidancecomplianceenforcement/complianceenforcement/bovinespongiformencephalopathy/default.htm>
- World Health Organization. Bovine Spongiform Encephalopathy
<http://www.who.int/mediacentre/factsheets/fs113/en/>

World Organization for Animal Health (OIE)
<http://www.oie.int>

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
<http://www.oie.int/international-standard-setting/terrestrial-code/access-online/>

OIE Terrestrial Animal Health Code
<http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/>

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