REVIEW

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Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: How safe is eating beef?

ABSTRACT

Cases of bovine spongiform encephalopathy (BSE, mad cow disease) have been found in North American cattle. Its human counterpart, called variant Creutzfeldt-Jakob disease (variant CJD), is rare but seems to be linked to eating diseased beef. Many questions remain about these diseases, such as why young people seem at greater risk of variant CJD. Also, are some people more genetically at risk for acquiring variant CJD than others?

KEY POINTS

BSE is a so-called prion disease. Although its origin is still disputed, it is thought to have been propagated by feeding cattle with meal made from the offal of infected sheep or cows.

Variant CJD occurs mostly in young adults (median age 29 years). It is marked by behavioral changes early on, followed by progressive cognitive impairment, helplessness, akinetic mutism, and death after a median of 14 months.

The neuropathologic features of variant CJD include spongiform changes, neuronal loss, and proliferation of astrocytes. A salient feature is numerous fibrillary amyloid plaques containing prion protein in the gray matter.

Prion diseases are believed to be caused by conversion of the host cellular prion protein into an abnormal infectious isoform that is resistant to protease.

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE), commonly known as mad cow disease, has appeared in the United States, raising concern about the possible appearance of its human counterpart, variant Creutzfeldt-Jakob disease (variant CJD). Several studies support the hypothesis that variant CJD is causally related to BSE and is acquired by consuming meat from diseased cows.[1,2]

We review the epidemiology and etiology of the human and bovine diseases, cover their clinical and pathological features, and discuss meat safety in the United States.

HISTORY OF BSE AND VARIANT CJD

Although the first case probably occurred several years earlier,[3] BSE was first diagnosed in 1985, when a dairy farmer in Kent, southeast England, noticed several cows with abnormal gait and altered behavior such as kicking unexpectedly during milking or aggressiveness (hence the name "mad cow disease").[4,5]

Findings in brain tissue from slaughtered cows were similar to those in scrapie, a spongiform encephalopathy that affects sheep.[5,6] More cases were diagnosed the following year, and a possible epidemic of a new disease in cattle was recognized.[3] Eventually, nearly 200,000 head of cattle had to be destroyed.

In humans, variant CJD was described in 1996 in the United Kingdom.[7,8] As of January 7, 2005, 159 cases have been reported: 148 in the United Kingdom, 7 in France, and 1 each in Canada, Ireland, Italy, and the United States (the Canadian, Irish, and US cases

were in people who had resided in the United Kingdom).[8]

BSE and variant CJD appear in North America

The spread of BSE to North America was first documented in 1993 when an imported cow tested positive in Canada. In May 2003, a cow born in Canada was diagnosed with BSE. The cow showed signs of the disease at slaughter, and Canadian officials prevented its meat from being sold for human consumption.[9]

In December 2003, an old Holstein cow in the United States tested positive for BSE after she arrived at a slaughterhouse near Mabton, Washington, on her knees.[9] (Cows that cannot stand are known in the meatpacking trade as "downers.") In January 2005, two additional cases of BSE were identified in Alberta, Canada.

NOVEL INFECTIOUS DISEASES

BSE and variant CJD are part of a group of fatal neurodegenerative diseases called transmissible spongiform encephalopathies, which can present as genetic, infectious, or sporadic disorders (TABLE 1). Ever since scrapie was first found in sheep a few centuries ago, various forms of these diseases have been reported in humans and animals.

BSE is thought to have spread via meal made from offal of BSE-infected animals

The currently favored theory of the etiology of these diseases, as proposed by Prusiner and colleagues,[10,11] centers around the prion protein.

The normal prion protein is a constituent of normal mammalian cells. Encoded by a gene on chromosome 20, it is predominantly found in the central nervous system, although small amounts are also present in other tissues. It may function as a receptor or adhesion molecule, has been linked to a signal transduction pathway, and may play a role in the metabolism of neurotransmitters.[12]

The molecular basis of prion diseases is believed to be the conversion of normal prion protein into the abnormal isoform, which is resistant to protease and infectious. Accumulation of the protease-resistant isoform in the central nervous system is thought to be responsible for neuronal loss and astrocytosis. Acquisition of protease resistance is a posttranslational process.[10,11]

The normal and abnormal proteins have the same amino acid sequence, but they differ in their three-dimensional structure. The normal protein has a high content of alpha helix (42%) and essentially no beta sheet (3%); the abnormal protein has an alpha helix content of 30% and a beta sheet content of 43%.[10,11]

Conversion from the normal to the abnormal protein is induced by aggregates and polymers of the abnormal protein and not by soluble, monomeric forms of it. Newly converted molecules become bound to the polymers. The conversion involves a conformational change in addition to the binding of the normal protein to the abnormal.[10,11]

Thus, the abnormal prion protein can be thought of as a novel infectious agent, one that lacks nucleic acid. The word "prion" by itself is often used to refer to the abnormal isoform, and the diseases that it causes are often collectively called prion diseases.

HOW DID BSE BEGIN?

Although the origin of BSE is still disputed, it is thought to have been propagated by feeding cattle with meal derived from offal (internal organs and other parts that would otherwise be wasted) from BSE-infected animals.[13]

Theory 1: BSE came from sheep

If BSE is the bovine form of scrapie, how did it get from sheep into cattle? The first known cases of BSE were at sites distant from one another in the United Kingdom, suggesting a general problem in cattle management rather than a local factor. It was fairly easy to rule out potential causes such as vaccines, biological agents, agricultural chemicals, and direct contact with sheep.[5] The only common factor identified was that the cattle had been fed meat-and-bone meal that contained ruminant-derived protein.[4]

In the early 1980s, increasing numbers of animals ended up as meat-and-bone meal at rendering plants, where protein is separated from fat to produce a protein-rich solid residue.[5] Although other countries had similar rendering systems, plants in the United Kingdom used a higher ratio of sheep to cattle tissue.[14] At the same time, new energy-efficient rendering techniques were adopted that

TABLE 1

Mammalian prion diseases

DISEASE	HOST	MECHANISM OF PATHOGENESIS
Kuru	Fore people	Infection through ritualistic cannibalism
Iatrogenic Creutzfeldt-Jakob disease (CJD)	Humans	Infection from prion-contaminated human growth hormone, dura mater grafts
Sporadic CJD	Humans	Possible somatic mutation or spontaneous conversion of normal prion protein into abnormal isoform
Variant CJD	Humans	Infection from bovine prions?
Familial CJD	Humans	Germline mutations in the prion protein gene
Gerstmann-Sträussler-Scheinker disease	Humans	Germline mutations in the prion protein gene
Fatal familial insomnia	Humans	Germline mutation in the prion protein gene
Fatal sporadic insomnia	Humans	Somatic mutation or spontaneous conversion of normal prion protein into abnormal isoform
Scrapie	Sheep	Infection in sheep
Bovine spongiform encephalopathy	Cattle	Infection with prion-contaminated meat and bone meal
Transmissible mink encephalopathy	Mink	Infection with prions from sheep or cattle
Chronic wasting disease	Mule deer, elk	Unknown
Feline spongiform encephalopathy	Cats	Infection with prion-contaminated bovine tissues or meat-and-bone meal
Exotic ungulate encephalopathy	Greater kudu, nyala, oryx	Infection with prion-contaminated meat-and-bone meal

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were less likely to destroy the highly resistant scrapie agent.[5,14–16]

In the 1970s, UK government policy encouraged farmers to produce more milk by removing calves from their mothers at an early age and feeding them meat-and-bone meal. Young cattle may be particularly susceptibility to infection with the BSE agent.[3–5]

Theory 2: BSE started in cattle

An alternative explanation for the origin of BSE is that it occurs sporadically (but very rarely) in cattle, and that tissues from an infected cow were incorporated into meat-and-bone meal to seed the epidemic. Although we have no evidence that BSE occurred in cattle before, it is possible that occasional cases arose without having been diagnosed.[14]

VARIANT CJD: FEATURES

TABLE 2 compares the clinicopathologic features of variant and sporadic CJD.[7,8,17–20]

Variant CJD occurs mostly in young patients: the median age at presentation is 29 years, compared with 65 years for sporadic CJD. The youngest known patient with variant CJD was 12 years old; the oldest was 74.

Most patients with variant CJD present with behavioral changes and are often first referred to a psychiatrist. Concomitant symptoms typically develop over the next several months and include depression, anxiety, withdrawal, forgetfulness, sensory symptoms, and ataxia. As in sporadic CJD, cognition becomes progressively impaired until the patient becomes completely helpless, and aki-

TABLE 2

Sporadic vs variant Creutzfeldt-Jakob disease (CJD)

FEATURE	SPORADIC CJD	VARIANT CJD
Mode of transmission	Autosomal-dominant in 10%	Consumption of meat
Median age at presentation	65 years or older	29 years (range 12–74 years)
Median duration of illness	4.5 months	14 months (range 6–39 months)
Genetics	Polymorphic at codon 129 of prion gene	Homozygous for methionine at codon 129 of prion gene
Electroencephalographic pattern	Generalized triphasic periodic wave complexes	Nonspecific slow-wave activity or normal
Cerebrospinal fluid 14-3-3 protein testing	Highly sensitive and specific	Less sensitive
Pathologic findings	Prion protein plaques in 5%-10%	Florid prion protein plaques
Abnormal prion protein in tissues outside the central nervous system	Spleen, muscle	Spleen, lymph nodes, tonsil, muscle, gastrointestinal tract
Course	Progressive, fatal	Progressive, fatal
Psychiatric symptoms	Present	Present
Neurologic signs*	Present	Present

*Unsteadiness, difficulty walking, involuntary movements

netic mutism often develops. Patients die a median of 14 months after symptoms appear (range 6–39 months).[17,20]

Diagnostic features of variant CJD

TABLE 3 summarizes the diagnostic criteria for variant CJD used in the United Kingdom.[21] A definitive diagnosis can be made premortem in a biopsy from the tonsil, spleen, or lymph nodes, or in a postmortem examination (see **Neuropathologic features**, below).

Electroencephalography shows nonspecific slow-wave activity, although it may be normal in some patients.

Magnetic resonance imaging typically shows increased signal intensity in the bilateral pulvinar nuclei.[20]

Cerebrospinal fluid protein testing. Protein 14-3-3 in the cerebrospinal nuclei is not elevated as often in variant CJD as in sporadic CJD. However, a higher percentage of tests have been positive recently compared with previous years,[17] possibly due to a change in the antisera used. In 2002 the antisera were changed from rabbit polyclonal anti-14-3-3y to mouse monoclonal anti-14-3-3-beta. When variant CJD samples from 2001 were reanalyzed using the new antisera, the sensitivity improved from 22% to 82% without significantly affecting specificity (92% vs 91%). The new test is not available at all hospitals but can be ordered from other laboratories.

Neuropathologic features associated with variant CJD include spongiform changes (FIGURE 1), neuronal loss, and proliferation of astrocytes.

A salient feature of variant CJD is numerous fibrillary prion protein amyloid plaques in the gray matter (FIGURE 1); in contrast, this occurs in only a minority of sporadic CJD cases.[21] Prion protein accumulation can be identified by immunohistochemistry of neural tissue and also of lymphoid tissue (spleen, lymph nodes, and tonsils). Some advocate using a tonsil biopsy in lieu of brain biopsy to confirm a diagnosis.[21,22]

The gold standard of diagnosis is demonstrating the abnormal protein by Western blot analysis.

TABLE 3

Diagnostic criteria for variant Creutzfeldt-Jakob disease

I	A	Progressive neuropsychiatric disorder
	B	Duration of illness > 6 months
	C	Routine investigations do not suggest an alternative diagnosis
	D	No history of potential iatrogenic exposure
II	A	Early psychiatric symptoms (depression, anxiety, apathy, withdrawal, delusions)
	B	Persistent painful sensory symptoms
	C	Ataxia
	D	Myoclonus, chorea, or dystonia
	E	Dementia
III	A	Electroencephalography (EEG) does not show the typical appearance of sporadic Creutzfeldt-Jakob disease (or no EEG performed)
	B	Magnetic resonance imaging shows increased signal intensity in the bilateral pulvinar nuclei
IV		Positive tonsil biopsy

Definite: Criterion IA (progressive neuropsychiatric disorder) plus neuropathological confirmation of variant Creutzfeldt-Jakob disease (spongiform change and extensive prion protein deposition with florid plaques throughout the cerebrum and cerebellum)

Probable: Criterion I (all 4 parts) plus 4 symptoms of criterion II plus criteria IIIA and IIIB, or criteria I and IV

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Needed: a comprehensive effort to map prion distribution in muscle of infected animals

Genetic studies. Recent studies have identified a genetic polymorphism in many cases. Of 133 cases of variant CJD examined, all have been in patients homozygous for methionine at codon 129 of the prion protein.[17,21]

HOW SAFE IS EATING BEEF?

Fortunately, variant CJD is rare. Although several lines of evidence indicate that it is acquired by eating BSE-infected beef,[1,2] much remains to be learned about the process.

Evidence that eating BSE-infected beef causes variant CJD

The biological and molecular transmission characteristics of variant CJD are consistent with its being the human counterpart of BSE.[1,23]

In 1999, Scott et al[23] reported on transgenic mice expressing bovine prion protein that serially propagate BSE prions. These mice were highly susceptible to variant CJD and natural sheep scrapie. When inoculated with either variant CJD or BSE brain extracts, their incubation times (250 days), neuropathology, and disease-causing prion protein isoforms were indistinguishable from each other and differed dramatically from those seen in mice injected with natural scrapie prions.

Furthermore, one might take in significant amounts of abnormal prion proteins through eating meat, even if it is largely free of neural and lymphatic tissue. In 2002, Bosque et al[24] found that muscle homogenates from infected mice, but not from uninoculated controls, displayed the abnormal prion protein of an apparent molecular weight and glycoform ratio identical to the abnormal prion protein found in the brain. There was much less of it in the muscle, however: the concentration was 500-fold lower than in the brain.

These observations indicate that a comprehensive effort to map the distribution of prions in the muscle of infected livestock is needed.

How safe is US beef?

Although cases of BSE are diminishing in the United Kingdom, cases are starting to appear in North America. Although only one domestic cow with BSE has been reported in the United States, testing is not uniformly per-

Variant Creutzfeldt-Jakob disease

Spongiform changes

Amyloid plaque

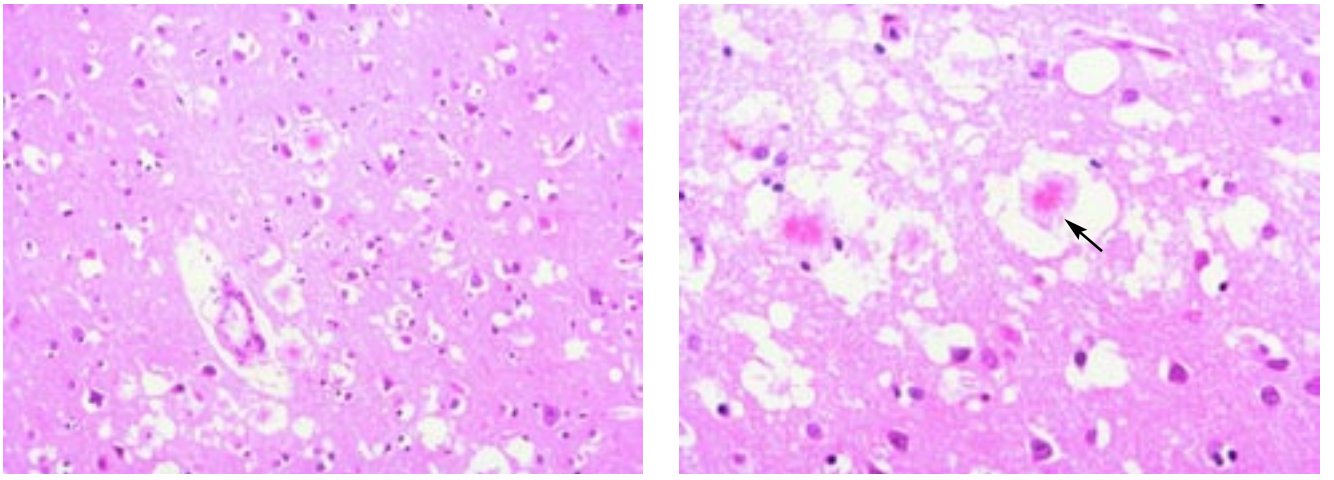

FIGURE 1. Brain tissue obtained at autopsy from a patient with variant Creutzfeldt-Jakob disease (variant CJD). **Left,** cerebral cortex with the characteristic spongiform changes, neuronal loss, and proliferation of astrocytes (hematoxylin and eosin, original magnification × 200). **Right,** Cerebral cortex with the typical fibrillary prion protein amyloid plaques in the gray matter (arrow) (hematoxylin and eosin, original magnification × 400).

formed: only suspicious cows (eg, downers) are tested. Some experts are concerned that routine screening of cattle is needed.

Muscle may provide a readily biopsied tissue from which to diagnose prion disease in animals without symptoms.[24] The efficiency of prion accumulation in muscle may vary with either the host species or the prion strain involved.

Cooking meat might not eliminate infection. The only reliable method for eliminating prions includes using chemicals (sodium chloride) and autoclaving at 134°C for at least 1 hour.

Why isn't variant CJD more common?

Oral transmission is inefficient compared with the intracerebral inoculations used for the bioassays in studies.

The species barrier must also be considered. In many cases, efficient transmission of prions from one species to another requires a high degree of homology in the amino acid sequence of the prion protein between the two species. The degree to which the amino acid sequence influences the efficiency of transmission depends also on the strain of prion.[24]

Cooking meat might not eliminate BSE infection

Many other questions remain. For example, why does the typical age of presentation of variant and sporadic CJD differ? What is the implication of the homozygosity at codon 129, and will it help determine the risk of developing variant CJD?

Hopefully, a better understanding of variant CJD will answer these questions and lead to prevention and treatment strategies.

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