

The Risk of Bovine Spongiform Encephalopathy ('Mad Cow Disease') to Human Health

Paul Brown, MD

Some human cases of the transmissible neurodegenerative disorder Creutzfeldt-Jakob disease recently seen in Great Britain are thought to have resulted from eating beef infected with the agent of bovine spongiform encephalopathy. Reasons for and against this presumption are explained, and the question of a similar situation occurring in countries other than Britain—in particular, the United States—is discussed in terms of the existence of scrapie (in sheep) or unrecognized bovine spongiform encephalopathy (in cattle), the practice of recycling nonedible sheep and cattle tissue for animal nutrition, and precautionary measures already taken or under consideration by government agencies.

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GREAT BRITAIN has played host to scrapie-infected sheep for the better part of 3 centuries, and scrapie was apparently introduced into cattle as bovine spongiform encephalopathy (BSE or "mad cow disease") a decade ago, through the feeding of recycled sheep carcasses in the form of meat and bone meal nutritional supplements.^{1,2} Now a serious question has arisen about its further spread to humans as Creutzfeldt-Jakob disease (CJD) from consumption of contaminated cattle products, because of the occurrence in Great Britain of 21 cases within a 3-year period of a "new variant" CJD syndrome (nvCJD) with unusual clinical and pathological features (Will et al³ and Robert Will, MD, unpublished data, August 1997). One additional case has also been reported in France, a major importer of British beef.⁴

SPONGIFORM ENCEPHALOPATHIES

Spongiform encephalopathies are a small group of fatal neurodegenerative

diseases that affect both humans and animals. Each disease has a separate name, sometimes an eponym to honor clinicians who first described it (eg, Creutzfeldt-Jakob disease), sometimes a descriptive term reflecting an important clinical feature (eg, fatal familial insomnia). All have the same underlying pathogenesis, and over the years they have been generically referred to as "slow and unconventional virus diseases" to indicate their unusually long course and virallike character; or "transmissible spongiform encephalopathies" and "transmissible cerebral amyloidoses" to emphasize their most characteristic neuropathologic and pathogenetic features; or the increasingly popular "prion diseases," anticipating the definitive demonstration of their causative agent as a proteinaceous infectious particle.

Creutzfeldt-Jakob disease was first described in the 1920s and is the most common human spongiform encephalopathy. It may be sporadic (random cases with no known cause), acquired from environmental sources (especially iatrogenic sources such as contaminated pituitary hormones or dura mater grafts), or familial (due to mutations in a gene on chromosome 20). It is respon-

sible for about 1 death per million people per year (200-250 cases each year in the United States). Other, rarer diseases are kuru (limited to a few adjacent linguistic groups in eastern New Guinea and now nearly extinct) and 2 exclusively familial disorders, Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia. All are experimentally transmissible.

In animals, spongiform encephalopathy occurs as a natural and fairly common affliction of sheep and goats (scrapie), as infrequent outbreaks in commercially bred mink, and as sporadic cases in wild or semicaptive ungulates. Under certain conditions, the disease may be transmitted from one animal species to another, of which the most recent and notorious example is the cow. The known and speculative interrelationships among these various diseases are illustrated in Figure 1.

CLASSIC CJD AND NEW VARIANT CJD

The classic sporadic variety of CJD usually appears in late middle age (average age, 60 years), but occasionally affects younger people, including adolescents. Typically, signs of the illness begin with loss of memory or confusion, but behavioral aberrations or gait instability are the presenting symptoms in about one third of patients. Later clinical features include a wide range of neurologic signs referable to the visual and motor cortex, basal ganglia, and cerebellum. Myoclonic or choreoathetoid movements associated with a distinctive electroencephalographic periodicity are seen at some time during the clinical course in most patients. The median du-

From the Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md.
Reprints: Paul Brown, MD, Bldg 36, Room 5B21, National Institutes of Health, Bethesda, MD 20892 (e-mail: pwb@codon.nih.gov).

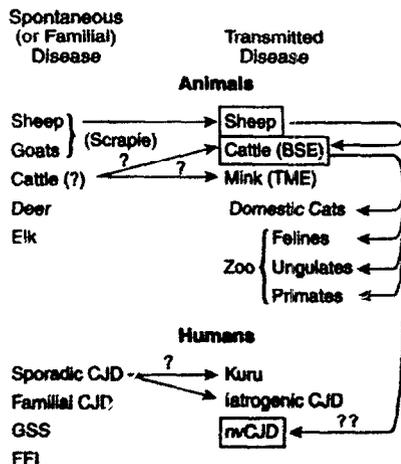


Figure 1.—Known and speculative interrelationships among the animal and human spongiform encephalopathies. CJD indicates Creutzfeldt-Jakob disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; FFI, fatal familial insomnia; BSE, bovine spongiform encephalopathy; nvCJD, "new variant" CJD; and TME, transmissible mink encephalopathy.

ration of illness is 4 months. At autopsy, microscopic sections of the brain show widespread spongiform change accompanied by gliosis and neuronal loss (Figure 2, left), and immunochemical staining reveals the presence of "prion" protein-positive amyloid deposits (Figure 2, right). In 5% to 10% of cases, these deposits are large enough to appear on routine staining as microscopically visible plaques (Figure 3, left).⁴

The recent cases of nvCJD are as a group remarkable for their comparative youth, all occurring in patients younger than 60 years (average age, 27 years); an onset with psychiatric and/or sensory symptoms; the absence of characteristic electroencephalographic changes; and an unusually long duration (average, 14 months). However, they share with sporadic cases of CJD the features of progressive dementia, ataxia, and myoclonus.^{2,8}

Cases clinically similar to nvCJD have occasionally been reported in pre-BSE national surveys of CJD, in Great Britain and elsewhere, so that 1 such patient among the 40-odd cases of CJD seen in Great Britain every year could easily have been dismissed as just another example of the clinical diversity of the disease. However, microscopic examination of their brains revealed hitherto unrecognized neuropathological characteristics dominated by myriad amyloid plaques surrounded by "petals" of spongiosis (so-called daisy or florid plaques) (Figure 3, right).^{2,8} The occurrence of multiple cases showing these unusual clinical and neuropathologic features within a period of 3 years caught the at-

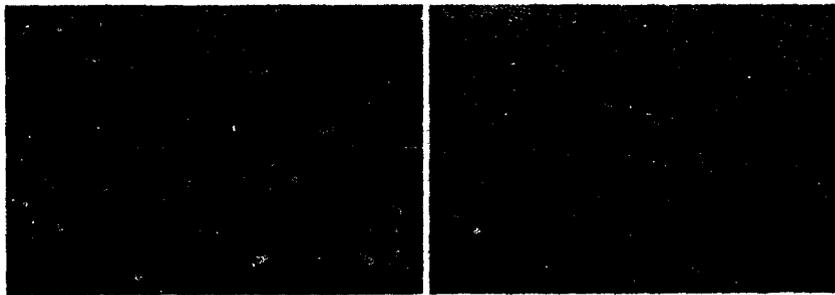


Figure 2.—Histological sections from the brain of a patient with sporadic Creutzfeldt-Jakob disease, showing spongiform (vacuolar) change (left) in a hematoxylin-eosin-stained section (original magnification $\times 80$) and "prion" protein-positive amyloid deposits (right) in an immunostained section (original magnification $\times 40$).

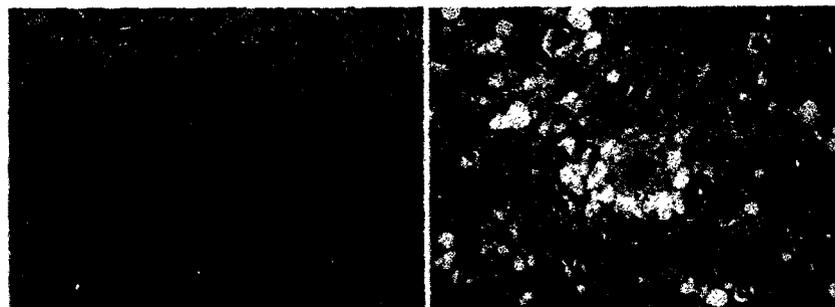


Figure 3.—Histological sections of amyloid plaques in sporadic Creutzfeldt-Jakob disease (left) and "new variant" Creutzfeldt-Jakob disease (right). Note the absence of surrounding vacuolation in the sporadic case and its presence in the new variant case (original magnification $\times 40$).

tention of physicians at the CJD Surveillance Unit in Edinburgh, Scotland, who surmised that they might have resulted from exposure to BSE.

nvCJD AND BSE

The new variant cases began to appear about 10 years after BSE was first identified in cattle, and we know from earlier studies that a 10- to 15-year incubation period typically intervenes between the infecting event and the onset of the symptoms of environmentally acquired CJD.⁷ All 21 patients were potentially exposed to contaminated bovine products during the 1960s, before the magnitude of the BSE problem was recognized and measures were taken to prevent human exposure. Also, their illness was in some respects reminiscent of kuru, which reached epidemic proportions as a result of the practice of ritual cannibalism, a parallel that invites consideration of an oral route of infection for these BSE cases.

Two experimental observations lend additional support for the idea that nvCJD could result from BSE infection: first, the inoculation of BSE-infected brain tissue into cynomolgus monkeys produces a spongiform neuropathology with daisy plaques very similar to those seen in the human nvCJD cases²; and second, electrophoretic analysis of the amyloid extracted from the brain tissue of patients with nvCJD shows a pattern similar to that of BSE-infected cows,

mice, and cats, but different from that of sporadic CJD.⁹

CONUNDRUMS

Three observations require explanation when considering BSE and nvCJD. First, scrapie-infected sheep have never been shown to have caused a case of CJD in humans anywhere in the world, despite the certainty that scrapie-contaminated sheep products have entered the human food chain on a nearly continuous basis for decades if not centuries. Because scrapie and BSE are essentially the same disease in 2 different animal species, why should BSE-contaminated products be infectious for humans when scrapie-contaminated products are not? Second, infectivity has never been detected in muscle or milk (the 2 most widely consumed livestock products) from animals naturally infected with BSE, scrapie, or any other spongiform encephalopathy. Third, if these products are consumed more or less equally by all elements of the British population, why have nearly all cases of nvCJD occurred in young people?

To each of these observations there are 1 or more caveats. The first is that no one can predict whether an infectious pathogen will change in the course of passing from one species to another (in this case, from sheep to cattle). For example, human brain tissue containing the kuru pathogen is capable of transmitting dis-

ease to ferrets only after passage through nonhuman primates (it does not transmit disease when inoculated directly into ferrets).¹⁰ Similarly, brain tissue from cows with BSE easily transmits disease to hamsters only after passage through mice.^{11,12} By analogy, therefore, scrapie might be pathogenic for humans only after passage through cows.

Second, our ability to detect very small amounts of infectivity is imperfect, and 1 or a few infectious particles present in a round of beef or a liter of milk could easily escape our tests, which depend on producing disease in healthy animals that are inoculated with extremely small amounts (30 μ L) of whatever tissue is being tested. As an example, the rigorous demonstration of the absence of infectivity in a 224-g (8-oz) steak would require the inoculation of nearly 10 000 mice—an experiment that will clearly never be undertaken. Also, muscle may be contaminated by central nervous system tissue, either during slaughter (killing by stun gun or "captive bolt" to the head of an animal may disperse brain tissue to other parts of the body, and halving a carcass with cutting instruments could disperse spinal cord tissue) or by the adulteration of muscle with spinal cord tissue in ground meat products.

The third observation concerning the occurrence of nvCJD in young people is more difficult to explain. It is possible that younger people are more susceptible to infection, or have a shorter incubation period than older people (in which case we may see increasingly older age groups being affected in the future).

We also should recognize that BSE-infected cattle are not the only possible source of infection of patients with CJD. Bovine spongiform encephalopathy is an obvious first choice because of its recent epidemic occurrence in an important livestock species, but other livestock animals such as pigs and poultry were also fed the same contaminated nutritional supplements, and even common garden fertilizers are made in part from rendered livestock bonemeal. We know that pigs can be experimentally infected by the intracerebral (but not oral) route with BSE, and although poultry have resisted both intracerebral and oral experimental infection with scrapie, there is no guarantee that they will prove to be resistant to BSE. Moreover, animals raised for commercial purposes are generally slaughtered at too young an age for disease to have become evident, so that the absence of epidemic disease in these species cannot be used as proof that they are not harboring the infectious agents. Even the existence of some healthy older hogs or chickens does not carry the weight of proof, because these

diseases affect only a small proportion of animals at risk—clinically evident BSE, for example, has occurred in fewer than 3% of British cattle after an epidemic period of 10 years. As for bonemeal fertilizer used in horticulture, the risk seems small, but not impossible.

DOES BSE OCCUR IN THE UNITED STATES?

Toward the end of the 1970s, both the US and British rendering industries changed their method of production from batch processing to continuous processing, and eliminated a processing step that would have reduced contamination by scrapie-infected carcass material: hydrocarbon solvent and live steam extraction of fat-soluble tallow from proteinaceous "greaves." This step is thought in retrospect to have been crucial for the prevention of scrapie (and, later, BSE) infectivity from being recycled to ruminants in the form of meat and bonemeal nutritional supplements.¹ Why, then, have we not seen a BSE epidemic in the United States?

One answer may be that strains of scrapie in the United States are sufficiently different from those in Great Britain as to be incapable of breaching the species barrier to cattle. Strain differences are a much studied phenomenon of spongiform encephalopathies, having been exhaustively defined in scrapie, and also recognized in CJD, kuru, and mink encephalopathy. Therefore, although British strains of BSE have shown an unexpected similarity to each other, there is no *a priori* reason to suppose that BSE in the United States (arising spontaneously in cattle or as the result of cross-over scrapie infection from sheep) would necessarily have the same biological characteristics as their British confreres.

Another explanation invokes the concept of an "infectivity threshold," postulating that the amount of infectivity in rendered material in Great Britain may have barely exceeded the threshold for disease transmission, and that an only slightly lower level of contamination would not have caused BSE. Compared with Great Britain, the United States has a smaller proportion of sheep-derived tissue in the mix of rendered animal protein (0.6% vs 14%) and an arguably smaller proportion of scrapie-infected sheep, although the precise incidence of scrapie in different countries is virtually impossible to determine. The reported annual incidence in the United States (30-50 cases per 8 million sheep) is similar to that in Great Britain (200-300 cases per 42 million sheep); however, the number of notified cases in Britain rose 2- to 3-fold during a period when slaugh-

ter was handsomely compensated (John Wilesmith, DVet, unpublished data, 1997), and in (admittedly imperfect) surveys of British sheep farmers, up to one third of flocks were said to be affected, with an in-flock incidence of 2%.¹³ Thus, relative to Great Britain, we have at least a 20-fold reduction in potential scrapie contamination based on the proportion of sheep infected with scrapie: alone or in combination, these factors might well result in a level of infectivity too low to transmit disease from sheep to cattle in the United States.

A third explanation is that we do have an epidemic, but have simply not recognized it. This assertion is based on 2 observations. The first (experimental) observation is that cattle inoculated with a US strain of sheep scrapie developed an ataxic neurologic illness without spongiform neuropathology that was diagnosed as BSE only by immunologic detection of "prion" amyloid in the brains of the dead animals.¹⁴ The second (epidemiologic) observation is that several outbreaks of spongiform encephalopathy have occurred in ranch-bred mink that were fed diets including (and in 2 instances apparently limited to) cattle parts or whole carcasses from "downer cows." Downer cows die of a variety of diseases that cause them to become sick and lie down (hence the name "downer"), and it has been suggested that unrecognized spongiform encephalopathy might account for some of the deaths.¹⁵ One further possibility is that BSE might exist as a silent infection that has not had time to manifest itself clinically (US cattle may be introduced to nutritional supplementation later and/or slaughtered earlier than British cattle, and so might have epidemic infection without disease).

Lest we be carried away by these speculations, it should be noted that pathologists in the US Department of Agriculture have examined more than 5500 brains from cattle dying of neurologic diseases since 1990 and, in a concurrent study, almost 1000 downer cattle since 1994, using both neuropathologic and immunohistological criteria, and have yet to identify a single case of spongiform encephalopathy. The same can be said for pilot studies in which either tonsils and/or brains from randomly selected cattle have been subjected to immunologic testing for prion protein. This is reassuring, and will be increasingly persuasive as additional thousands of animals are examined in coming years. Also reassuring is the fact that humans with the distinctive features of nvCJD have not been identified in the United States either in current surveillance studies or on review of clinical and neuropathologic hospital archives from the last decade.¹⁶

RISK ASSESSMENT

Although we cannot now undo the consequences to human health of past exposure to BSE, we must continue to clarify the risk of that exposure and, in particular, to obtain evidence for or against the presumptive connection between BSE and nvCJD. From the laboratory, we can expect continued electrophoretic analyses of amyloid extracted from the brains of patients with nvCJD and sporadic CJD to provide "fingerprints" of incrimination or exoneration of BSE. We also await the results of independent fingerprint studies based on the inoculation of brain tissue from patients with nvCJD into a panel of mice known to have different incubation period and brain lesion topography patterns for different strains of spongiform encephalopathy. If these strain typing results accord with electrophoretic analysis studies, the validity of a link between BSE and CJD will be much strengthened. Furthermore, although we know that marmoset and cynomolgus monkeys can be infected by intracerebral inoculation of the BSE agent, it would be more appropriate to know if a primate species more closely related to humans (eg, the chimpanzee) living on a diet that included repeated feeding of meat and milk from BSE-infected cows (ie, duplicating the human situation) would develop disease. Comparatively inexpensive alternative test animals might be transgenic mice carrying the human prion gene, which have already proved useful as assay animals for the diagnosis of sporadic CJD.

Because the laboratory can only provide clues to the problem posed by nvCJD, we must turn to classic field epidemiology for a definitive answer: only through continued surveillance of CJD in Great Britain and other countries (regional surveillance has just been initiated in the United States) will the suggested connection between BSE and

CJD eventually be proved or disproved. If cases of nvCJD result from consumption of BSE-contaminated tissue, they may represent a limited group of susceptible individuals within a British population that has been exposed to very low doses of the infectious agent, or (less likely) they may represent the first cases of an evolving epidemic of unknown proportions. Statistical modeling suggests that it may take 2 to 3 more years of observation before the alternatives become clear.¹⁷

PRECAUTIONARY MEASURES

As the BSE epidemic unfolded, the British government instituted a series of measures designed to minimize the risk for disease transmission among both animals and humans. These included the compulsory slaughter and destruction of suspect cattle, a ruminant-to-ruminant feed ban, elimination of certain high-risk organs such as brain, spleen, and thymus from the animal and human food chain, a ban on mechanically recovered meat from bovine vertebral column for human food, and, most recently, the removal of cattle older than 30 months and the heads of cattle older than 6 months from all food chains.²

In the United States, concern about the possible present (or future) existence of BSE has provoked numerous discussions between the Food and Drug Administration, the Department of Agriculture, sheep and cattle farmers, and the rendering industry to consider sourcing and processing safeguards to prevent an outbreak of disease. Several precautionary measures have already been taken, and it is likely that additional regulations will be forthcoming. A continuing embargo was instituted in 1969 on the importation of all cattle and cattle products originating in Great Britain or other countries with possibly indigenous BSE, and cattle imported during the 1980s before the embargo was put in place were traced and

either put under surveillance or slaughtered. With respect to indigenous scrapie, the Department of Agriculture has placed restrictions on the movement of sheep from infected to uninfected flocks, and encouraged a "flock certification" program for the sheep farming industry, in which the documented absence of scrapie during a preceding 5-year period will be officially recognized with a view to promoting a premium price for such animals, and thereby provide an incentive for reducing the incidence of scrapie in this country.

Under present scrutiny are a number of measures to improve the safety of the rendering and animal feed industries, including a return to the pre-1980 processing method that used hydrocarbon solvent and steam extraction steps, and a variety of feed exclusion proposals. After considerable professional consultation and several public hearings, the Food and Drug Administration issued a regulation effective June 1997 instituting (with certain exceptions) a mammal-to-ruminant feed ban. It is possible at some future date that the recommendation could extend to the total elimination of recycled animal tissue for livestock nutrition, and, despite the absence of convincing evidence for the existence of BSE-affected cattle in the United States, could also consider restrictions on high-risk tissues (brain, etc) for human consumption. Finally, the question of potential risk posed by bovine-sourced biological products ranging from myelin (used in the treatment of multiple sclerosis) to gelatin (used in products from drug capsules to cosmetics), will need to be evaluated. Altogether, a full plate for government regulatory agencies.

This article is dedicated to the memory of Richard Marsh, DVM, who early cautioned us about accepting scrapie-infected sheep as the sole source of transmissible spongiform encephalopathy, and sounded a warning about the potential danger inherent in the recycling of animal tissue for nutritional purposes.

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