Director's Digest

FATS AND PROTEINS RESEARCH FOUNDATION, INC.

#275

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January 1996

Bovine Spongiform Encephalopathy (BSE):
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Introduction.

Since bovine spongiform encephalopathy (BSE) was originally diagnosed in 1986, there were obvious questions that pertained to the possible transmission of the causative infectious agent to humans through the consumption of beef or beef products. A decade later, the questions persist devoid of finite answers or a clear scientific validation of proof to support or refute the potential for transmission. The complexity of the disease neither permits ready nor easy answers, thus permitting a large cohort to indulge in a point-counter point and heighten their biases. I venture my perceptions based on a retrospective analysis of the relevant literature and plead continued caution because the biology, pathogenesis, and transmission of the spongiform encephalopathies in animals and man is intricate and challenges the ingenuity of all who have taken the time to assess the implications of the disease. Nonetheless, a plethora of information exists to permit assumptions and inferences from the published references.

Bovine spongiform encephalopathy (BSE) is a fatal degenerative disease of the central nervous system (CNS) of cattle, first recognized clinically in Great Britain. (1) Some clinicians allege that they saw signs/symptoms of the disease in cattle varying from months to a year prior to official histopathologic confirmation of the disease. One researcher has described BSE as the first subacute spongiform encephalopathy identified in cattle, and the bovine equivalent of scrapie in sheep. (2) The disease belongs to a group of related neurologic diseases commonly known as the transmissible spongiform encephalopathies (TSEs). The group includes scrapie, which

affects sheep and goats; transmissible mink encephalopathy; feline spongiform encephalopathy; chronic wasting disease of mule deer and elk; and three rare diseases in people: kuru, Creutzfeldt-Jakob Disease (CJD) and Gerstmann-Straussler-Scheinker (GSS) syndrome. (3)

The incubation period of the disease varies from two to eight years. The majority of the cases in Great Britain occur in dairy cattle or in dairy crossbreds in their productive years - between three and five years old. (1,3) The causative agent of the disease is unknown. The prevailing theory, based on histologic findings, is that the infectious agent is identical to or similar to the yet unidentified agent that causes scrapie in sheep and goats. (4) Epidemiologists in Great Britain have implicated the source of the BSE agent to be meat and bone meal (MBM) from scrapie-infected sheep used as a protein supplement in feeds for young calves and lactating cows. (2)

Any discussion of etiology associated with either slow viral infections of the central nervous system (CNS) or the unconventional infectious agents causing slow infections compels a review of the history of investigations of these diseases to heighten both an understanding and appreciation of the genesis and continued progress made in research endeavors over the years. Research work done in Iceland by Bjorn Sigurdsson in the 1940s on visna, an epidemic disease of the nervous system and maedi, a slow progressive pulmonary pneumonia of sheep, provided the initial discovery of a new class of slow infections characterized by a long incubation period, heightening the concept of slow infections. (5) In 1957, shortly after the concept of slow infections became established, Gajdusek and Zigas described a degenerative neurologic syndrome, kuru, seen in the Fore tribe that inhabited the highlands of New Guinea. (6) Extensive research by Hadlow on the pathologic characteristics of scrapie demonstrated marked similarities to kuru. This resulted in further investigations that ultimately established the slow infectious nature of kuru. (7,8) Further comprehensive studies on the transmission of the infectious agent to chimpanzees documented the infectious basis of Creutzfeldt-Jakob disease (CJD), a degenerative dementia, firmly establishing the pertinence of slow infections to human medicine. (9)

The Prion Concept.

The diseases in animals and humans caused by unconventional agents have unusual properties. They are filterable and replicate like viruses, but they differ markedly from viruses in their resistance to physical and chemical treatments that will normally inactivate conventional viruses and failure to elicit an immune or inflammatory response. (10) A distinct structure first observed by Merz and coworkers and associated with the replication of unconventional agents in the brains of scrapie-infected mice was designated scrapie-associated fibril (SAF), an amyloid protein. (11) SAFs are traditionally found in diseases associated with unconventional agents and have become a significant distinguishing factor in the diagnosis of these complex

diseases. Prusiner and coworkers isolated the major protein of SAFs in 1982 and suggested in research findings that the scrapie associated protein (SAP), later abbreviated PrP (prion protein), is the molecule that transmits infection of the transmissible spongiform encephalopathies (TSEs). (12)

The clinical signs (BSE)

The onset of clinical signs is insidious. The disease progresses until the affected animal inevitably dies, usually in one to six months. In the early stages, the animal appears apprehensive and fearful. Later, the animal exhibits erratic reactions to sound and touch, as seen in patients with rabies. Many early signs are suggestive of metabolic or deficiency syndromes, such as nervous ketosis and hypomagnesemia, or toxic conditions, such as ryegrass staggers. (13,14) The animal's gait is uncoordinated and characterized by a loss of balance and swaying, often with high-stepping, particularly of the hind feet. Some cows may paw the ground or lick their nostrils frequently. The general posture is abnormal, Tremors, an abnormal head carriage, and knuckling at the fetlock are present. Affected animals are afebrile and exhibit progressive deterioration with loss of body weight despite a normal appetite, reduced milk yield, ataxia, and falling. (13,14,15)

Creutzfeldt-Jakob Disease (CJD)

Creutzfeldt-Jakob disease (CJD) is a presenile dementia found throughout the world, and except in rare instances in which the agent was inadvertently transmitted like in a contaminated corneal implant, the mode of transmission of the disease is UNKNOWN. (5,10) CJD patients usually display memory loss, abnormal behavior, changes in personality, and impaired cognition and judgment. The mental deterioration accompanied by other central nervous system (CNS) manifestations progress rapidly from month to month, terminating in death within 4-7 months of onset. In reality, 90 per cent of patients die within a year. CJD belongs to the broad group of transmissible spongiform encelphalopathies (TSEs)/neurodegenerative diseases seen in animals and humans, and is the most significant human prototype of the prion diseases. (5,10) This disease will be the designated reference for the public health inferences to bovine spongiform encephalopathy (BSE).

The public health pertinence.

Since no peer review journal has published a comprehensive analysis of a possible associative link between bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) because of the complicated pathogenesis, unknown transmission, and prolonged incubation period, a thorough evaluation of the science is impossible. An assessment, however, can be made of the media coverage of the diseases, and premises and conclusions can be discussed with relevance. It is

absolutely essential to state from the inception that bovine spongiform encephalopathy (BSE) as described clinically and histopathologically in the United Kingdom (U.K.) Does not exist in the United States. That has been accepted as fact by animal health authorities throughout the world. One obvious question at this juncture ought to be why after the existence of the disease in the U.K. for about a decade has the United States been "exempt."? There could be several answers to this analogy, but I will limit my reply to the differences of the existing risk factors between the two countries. Marsh (16) reported that the risk of BSE occurring in the United States is low...(our lower prevalence of scrapie, smaller population of sheep, and present practice of feeding meat and bone meal to mainly lactating animals indicate that our exposure is less than Great Britain). We addend Marsh's observations to highlight the limited risk; the government's active scrapie control program in the United States; the lower incidence of scrapie in the United States; the limited amount of sheep material used in the United States (.00183% of total rendered material) markedly reduces the scrapie titer to non-infectivity; the Animal Protein Producers Industry (APPI) voluntary control program prohibiting the use of rendered sheep-goat carcasses for use in cattle or other ruminant feeds; and the possibility that the scrapie "strain"/isolate in the United States is different from the U.K. isolate. These factors clearly heighten the current controls that limit the potential risk of BSE occurring in the United States, thus obviating the public health significance.

A most interesting aspect of scrapie/BSE is ongoing research in the United States administered by the Agricultural Research Service (ARS) of the United States Department of Agriculture since September 1991, in which 24 experimental calves (4 Holstein Friesian and 20 Jersey) starting at 4 to 6 weeks of age were fed meat and bone meal (MBM) and tallow that were prepared from sheep with a high incidence of scrapie in the flocks. Brains from some of these sheep were examined histologically and found to have lesions of scrapie. Some 550 gallons of each meat and bone meal (MBM) and tallow were fed at approximately 6% and 3% respectively of the ration to completion. Twelve of the original steers were sacrificed in February 1992 and histologic examinations revealed no signs, lesions, or PrP indicative of disease. The remaining twelve steers, approximately 5 years after the commencement of the research are normal. (Personal communication, 1-25-96, Ames, IA.) (17)

An in-depth analysis of the following publications provides a perspective of the frustration (s) in making reasonable/sensible inferences from media coverage.

1. Vancouver Sun, December 4, 1995.

A well-known British physiologist, Prof. Colin Blakemore, said that eating meat is "just not worth the risk. There is growing evidence of a transmission to humans." No proof, devoid of substance, definitely no science. The most interesting aspect was that he did

not concur with the British government's assurances that BSE could not pass to humans through beef consumption.

2. New York Times, January 12, 1996 - fear of Mad Cow Disease Spoils Britain's Appetite.

Robert Lacey, a microbiologist of Leeds University was quoted "We know in general that most of these infectious agents can go from one animal to another." If Professor Lacey is implying "spread" or contagion among cattle as a potential in disease transmission, this is contrary to all the current scientific evidence. All epidemiologic indicators have clearly demonstrated that this is not so and that BSE is a perfect example of a "dead-end disease."

The article continued by alleging that in Britain "Cows at the slaughterhouse get only a quick visual inspection. The government has so far refused to do sample testing for mad cow disease." This form of obvious misinformation heightens public apprehension and does a great disservice to the efforts of government regulatory officials, and, indeed, to the cattle industry and meat processors of the U.K. The fact is that there are no available routine laboratory methods for the ante-mortem confirmation of BSE. Equally relevant, the disease is diagnosed clinically by signs clearly recognized as unusual. These signs/symptoms are readily discerned by livestock handlers. In reality, this is the manner that most cases of BSE were diagnosed initially.

Confirmation of the disease is through laboratory assessment of histopathological changes/lesions of the central nervous system (CNS), and detection of prion protein (PrP) by immunoblotting or immunocytochemistry - or molecular pathology. The diagnosis of BSE is definitely not a kill floor regimen. Yet, the disinformation persists causing the potential for anxiety on an unsuspecting already confused public. The government of Great Britain has maintained a program of informational updating by routinely reporting all newly confirmed cases of BSE, and conducts extensive surveillance and research, reporting all findings to interested parties without reservations. The U.K. authorities continue to inform the public that there is no associative link of BSE to CJD. The science suppports the pronouncements by the government.

(Government authorities in the United States, a country in which BSE does not exist, through the National Veterinary Services Laboratories, report routinely on the BSE surveillance program. Approximately 2,500 brains have been submitted for examination, without evidence of the disease.)

3. New York Times, Letter to the Editor, January 16, 1996 - Blame Factory Farming for Mad-Cow Disease.

Dr. David Ehrenfeld of Rutgers University in his letter to the editor stated that the disease (BSE) is spreading among cattle in Britain. On the contrary, according to the epidemic curve there is a continued steady decline in the incidence of the disease. The letter continued by suggesting that maybe Creutzfeldt-Jakob disease (CJD) should be listed as a side effect of factory farming. Again, a plethora of irresponsible statements highlighting negatives that are totally devoid of scientific references. And, a grave injustice to cattle farmers, meat processors, and the regulatory agencies responsible for inspection and the health and welfare of the public.

4. The Toronto Star, January 14, 1996.

This publication also heightened statements predicted by Prof. Lacey from a more detailed perspective to demonstrate the utmost lack of responsibility in some of the public pronouncements carried by the media. "By the year 2010 between 5,000 and 500,000 Britons would die annually as a result" of the alleged BSE relevance to CJD. This type of statement gets the maximum attention of the media without scientific rationale. Note the numerical spread difference of the death predictions. It is imperative to return to a rational assessment of the existing science - the specific cause of BSE and CJD remains unknown. There is no proven associative link of BSE to CJD; on the contrary, the official government committee of the U.K. responsible for assessing the public health risk maintains "there is...no need to take extreme measures with respect to the British beef that is allowed to enter the food chain." Nevertheless, speculate on the impact when death in the thousands is predicted by someone having a university appointment, and doubt and confusion becomes commonplace. The debate is obviously accelerated, not in the context of scientific and investigative research, but sheer emotionalism.

The above extrapolations of four examples of media coverage are very representative of the type of reporting on the subject. This provides a distinct challenge to the scientific community to counter emotional outbursts and unsubstantiated predictions with the findings of science. It is equally distressing to observe that the official government pronouncements of no harm and no linkage of BSE to CJD are given only cursory mention by the media.

The transmissibility of CJD was first demonstrated in 1968, and scrapie was epidemiologically considered a possible causal prototype to this disease. Research conclusions were that "sheep are not the natural reservoir for CJD infection and there is no evidence to implicate any other animal species." (18) "It is a curious fact that the general public, which has become excited about the possible danger from BSE, has never expressed any concern about exposure to scrapie, a vastly more widespread disease with a venerable history of entrance into the human food chain." (19) "The global epidemiology of CJD is that of a randomly disperse disease with an annual overall incidence of about 1 case per 2 million people, usually higher in urban than

rural areas." (20,21) "Statistical analysis of the tempro-spatial distribution of CJD has revealed no case aggregates that could not be explained by chance alone." (19)

Brown (19) in his conclusions examining the pertinence of BSE and CJD based on clinical epidemiology "see little or no danger from the more commercially important consumption of meat and milk products, which contain no detectable infectivity." Adjunctly, extensive national epidemiologic surveys in England, Israel and Japan found no relationship between diet and the frequency of CJD. (19)

According to Will (22) "the risk of transmission of BSE to the human population is likely to be remote.....the risk of oral transmission by the consumption of tissues unlikely to contain significant titres of the infectious agent, such as muscle or milk is remote." The statements of Will were based on experimental and epidemiological evidence supporting the prevalent scientific view that BSE as a risk to the human population is remote.

Professor J. Collinge of Britain's Imperial College School of Medicine in research published in the December issue of Nature, "Unaltered susceptibility to BSE in transgenic mice expressing human prion protein" demonstrated the inability of bovine aberrant PrP to affect normal human PrP. The research involved mice genetically engineered to produce human prion protein (PrP) as well as normal mouse prion protein (PrP). The mice were injected with the infectious agent of CJD, and the mice succumbed, clearly demonstrating that with human protein, the human disease CJD was transmitted. Similar mice were then exposed to BSE. Abnormal mouse prion protein was formed, but no abnormal human prion protein. (23) The "species barrier" will vary from disease to disease, and will vary between different animal species and between a given species and humans. Collinge's work provides a clear indication that for BSE, the species barrier between cattle and humans is much greater and definitely harder to cross compared to between cattle and mice.

Another interesting epidemiologic aspect is that CJD occurs more or less in a very uniform manner throughout the world, regardless of the occurrence of BSE or scrapie. In Europe, the highest incidence of CJD is in the Netherlands (no BSE) at 1.04 cases per million person-years. This should be compared to the U.K. (With BSE) with an incidence of 0.93 cases. Because CJD is rare and does not permit for reliable and extensive epidemiological studies, some interesting facts need to be examined for comparative relevance, e.g., why do clergymen and professional drivers suffer "above-average" CJD incidence? Also in spite of inferences to the contrary, there is no statistical or scientific evidence that many of the occupationally exposed like butchers, veterinarians, slaughterhouse workers, and farmers have a higher than average incidence of CJD. (24)

Government experts, independent researchers and specially appointed

committees have provided opinions that were endorsed by the House of Commons Agriculture Committee and the Southwood Commission that the chance of man becoming infected through meat or milk from BSE infected cattle were extremely remote. The United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF) in a public statement on November 13, 1995, provided information that endorsed the control efforts of the Ministry. The World Health Organization (WHO), the International Veterinary Organization (OIE), and the European Commission (EC) Scientific Veterinary Committee all stated that measure taken by the government were recognized as being sufficient. The independent Spongiform Encephalopathy Advisory Committee (SEAC), the advisory body to the U.K. government on all aspects of BSE and CJD endorsed the ongoing control measures and stated again that in the committee's opinion there was no evidence of a link between BSE and CJD. (25) The Chairman and Vice-Chairman of SEAC in an open letter (December 13, 1995) stated that if there ever were any risk to human health from BSE, and there may be none, it was very much less in December 1995 than it had ever been. (25)

In a number of published research papers, tissues and secretions from confirmed BSE cattle were fed or injected into mice, and, with the exception of brain and spinal cord, all have failed to transmit the disease. This heightens the opinions of several researchers that milk and meat products do not carry the infective agent. (25) On January 11, 1996, Independent Television News (ITN) of Great Britain reported on the work/findings of the CJD Surveillance Unit at Edinburgh which stated "Deaths from CJD were not thought to relate to BSE. World-wide, CJD occurred naturally at a more-or-less constant rate regardless of whether BSE occurred at all. Comparing in-brain development characteristics of BSE with those of CJD did not indicate any link." (25)

Discussion/Summary

Bovine Spongiform Encephalopathy (BSE) is a complex disease, characterized by a long incubation period and a causative agent that has never been identified. The same can be said for Creutzfeldt-Jakob disease (CJD). The world of Science reacts to BSE based on the hypothesis that the disease resulted from the consumption by cattle of meat and bone meal (MBM) containing a scrapie-like infectious agent. This is not conclusive. It is suppositional. There have been reports of animals that have died of BSE in Great Britain that supposedly were never fed MBM. Since "time immemorial" we have been feeding MBM in the United States to cattle, this begs the obvious question, why have we not had a case of BSE? The prevailing opinion by government epidemiologists is that the risk factors for BSE do not exist in the United States. This theory is based on an extensive risk analysis published by the Animal and Plant Health Inspection Service (APHIS), Centers for Epidemiology and Animal Health (CEAH) in Fort Collins, Colorado.

A risk to humans consuming beef would depend on two basic conditions, first

that BSE could be transmitted from cows to humans, and second, that parts of the animal capable of carrying the infectious agent could enter the human food chain. As to the first, there is no scientifically validated evidence that BSE can be transmitted from cows to humans, and there is ample research evidence that it cannot. As to the second, muscle meat and milk have been shown to be incapable of carrying the infectious agent. Yet, in spite of the preponderance of evidence based on extensive scientific investigations, there are still many who maintain doubts and are downright suspicious of government's claims of safety of beef. Also, the epidemiologic findings that CJD also occurs in life long vegetarians!

Research work by Presiner on scrapie and Collinge with BSE clearly demonstrates strong evidence that the species barrier for prion disease between animals and humans was much harder to cross than those between animals. These type of research initiatives need to be pursued because they have started to provide a significant and important body of information about these complex diseases of animals and man. The elusive nature of these diseases could readily be exemplified by scrapie, a central nervous system (CNS) disease, well described in the veterinary/medical literature in England and Germany over 100 years ago, with a causative agent still to be identified. Thus, simplistic statements that BSE has a link to CJD harm the scientific agenda and put the debate into the hysterics and emotionalism of the media, doing a grave injustice to government control and research effort.

The accomplishments of research initiatives in industrialized societies have provided answers to difficult issues challenging the veterinary and medical professions in the past. BSE and CJD again provide ample opportunities to both groups to work collaboratively to find answers to these complex diseases that mock our ingenuity. BSE has presented us with a novel neurodegenerative disease related to a poorly understood type of transmissible infectious agent. CJD, in contrast, has been well defined and epidemiologically described in the medical literature for years, but, like BSE, the causative agent remains unknown, and, therein, lies the challenge and the problem. Our priorities must include continued epidemiological studies of the animal and human transmissible encephalopathies, molecular biological studies to develop a test that accurately detects infection in the live animal, and development of improved strain typing methods to assist epidemiological tracing. A global approach is necessary, and working in concert could assist the impetus to find the answers to these difficult to understand diseases.

REFERENCES

1. Franco, D.A.: Preventing an outbreak of bovine spongiform encephalopathy. Vet. Med. 3: 254-261, 1994.

2. Wilesmith, J. W. et al. : Bovine Spongiform Encephalopathy: Epidemiological Studies. Vet. Rec. 123: 638-644, 1988.

3. United States Department of Agriculture, APHIS, Bovine Spongiform Encephaolpathy, Fact Sheet, 1990.

4. Walker, K. D. et al: Comparison of Bovine Spongiform Encephalopathy Risk Factors in the United States and Great Britain. JAVMA, 199 (11): 1554-1561, 1991.

5. Haase, A.T.: Slow Virus Infections of the Central Nervous System. In: Infectious Diseases; Gorbach, S.L., J.G. Bartlett, and N.R. Blacklow (Eds), W.B. Saunders, Phila., 1206-1216, 1992.

6. Gajdusek, D.C. and Zigas, V.: Degenerative disease of the central nervous system in New Guinea. N. Engl. J. Med. 257: 974. 1957.

7. Hadlow, W.J.: Scrapie and kuru. Lancet, 277:289, 1959.

8. Gajdusek, D.C., Gibbs, C.J. Jr., Alpers, M.: Experimental transmission of a kuru-like syndrome to chimpanzees. Nature. 209:794, 1966.

9. Gibbs, C.J. Jr., Gajdusek, D.C., Asher, D.M. et al.: Creutzfeldt-Jakob disease (spongiform encephalopathy): Transmission to the chimpanzee. Science. 161:388, 1968.

10. Haase, A.T.: Unconventional Agents Causing Slow Infections: Creutzfeldt-Jakob Disease and Kuru. In: Infectious Diseases, Gorbach, S.L., J. G. Bartlett and N.R. Blacklow (Eds), W. B. Saunders, Phila. 1865-1873, 1992.

11. Merz, P.A., Rohwer, R.G., Kascsak, R. et al: Infection-specific particle from the unconventional slow virus diseases. Science. 225:437, 1984.

12. Bolton, D.C., McKinley, M.P., Prusiner, S.B.: Identification of a protein that purifies with the scrapie prion. Science 218:1309, 1982.

13. Anon. Bovine Spongiform Encephalopathy. Disease Update. Vet. Rec. 122:477-478, 1988.
14. Wells, G.A.H. et al.: A Novel Progressive Spongiform Encephalopathy of Cattle. Vet. Rec. 12 (1): 419-420, 1988.

15. Cranwell, M.P. et al.: Bovine Spongiform Encephalopathy. Vet. Rec. 122:190, 1988.

16. Marsh, R.F.: Risk Assessment On the Possible Occurrence of Bovine Spongiform Encephalopathy in the United States. Proceedings of a Seminar, Sub-Acute Spongiform Encephalopathies (Bradley, R.; Savey, M., and Marchant, B. Eds.) Brussels, Belgium, 1990.

17. Miller, J.: Personal communication. (1-25-96)

18. Kimberlin, R. H.: Review of Scrapie-Like Diseases to 1986. Proceedings of a Seminar, Sub-Acute Spongiform Encephalopathies. (Bradley,R., Savey, M., and Marchant, B. Eds. 1-9., Brussels, Belgium, 1990.

19. Brown, P.: The Clinical Epidemiology of Creutzfeldt-Jakob Disease In The Context of Bovine Spongiform Encephalopathy. Proceedings of a Seminar, Sub-Acute Spongiform Encephalopathies. (Bradley. R., Savey, M., and Marchant, B. Eds. p. 195-202, Brussels, Belgium, 1990.

20. Masters, C.L., Harris, J.O., Gajdusek, D.C., Gibbs, C.J. Jr., Bernoulli, C., and Asher, D.M.: Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering. Ann. Neurol., 5, 177-188, 1979.

21. Brown, P., Cathala, F., Castaigne, P., and Gajdusek, D.C.: Creutzfeldt-Jakob disease: clinical analysis of a consecutive series of 230 neuropathologically verified cases. Ann. Neurol., 20, 597-602, 1986.

22. Will, R.G.: Is There A Potential Risk of Transmission of BSE to The Human Population And How May This Be Assessed? Proceedings of a Seminar, Sub-Acute Spongiform Encephalopathies (Bradley, R., Savey, M., and Marchant, B., Eds. Brussels, Belgium, 179-186, 1990.
23. Collinge, J. et al.: Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. Nature. 378, 21-28 December. 1995.

Taylor, D.M. et al.: Archives of Virology, 139, 313, 1994.

Smith, P.E.M. et al.: CJD in a Dairy Farmer., The Lancet, 346, 30, September, 898. 1995.

24. and 25. Internet references/extrapolations from:

Barlow, R.M. et al: Veterinary Record, 126, 111, 1990.

Frazer, H. et al: Journal of General Virology, 73 1891, 1992.