

**UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MONTANA
BILLINGS DIVISION**

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RANCHERS CATTLEMEN ACTION LEGAL FUND)	
UNITED STOCKGROWERS OF AMERICA,)	
)	
Plaintiff,)	
)	
v.)	
)	
UNITED STATES DEPARTMENT OF AGRICULTURE,)	Cause No.CV-05-06-BLG-RFC
ANIMAL AND PLANT HEALTH INSPECTION)	
SERVICE, et al.,)	
)	
Defendants)	
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DECLARATION OF STANLEY B. PRUSINER, M.D.

Stanley B. Prusiner, M.D. certifies and states as follows:

1. I submit this declaration in connection with R-CALF USA’s motion for summary judgment in the above-captioned action. It is based on my own personal research and on my familiarity with research performed by others, including the scientists working in the laboratory that I oversee.
2. I am the Director of the Institute for Neurodegenerative Diseases and a Professor of Neurology and Biochemistry at the University of California, San Francisco (UCSF). I am also the founder of InPro Biotechnology, Inc.
3. In 1997, I was awarded the Nobel Prize in Physiology or Medicine for my discovery of the novel protein agent, which I named “prions,” that are the cause of

transmissible spongiform encephalopathies (TSEs), or prion diseases, such as bovine spongiform encephalopathy (BSE).

4. I am a member of a number of learned societies including the National Academy of Sciences, the Institute of Medicine, the American Academy of Arts and Sciences, and the American Philosophical Society. I am also a foreign member of the Royal Society, London.

5. I am the editor and a contributing author of *Prion Biology and Diseases*, which was published in 1999 to encourage young scientists to enter the field of prion biology, as well as to serve as a reference source for senior investigators. The second edition of *Prion Biology and Diseases* was published in 2004 to document the substantial advances that have been made in prion research since 1999. In addition to *Prion Biology and Diseases*, I am the editor of ten books and an author of hundreds of scientific articles in peer-reviewed journals and book chapters. A complete list of my publications is contained in my curriculum vitae which is attached to this declaration as Appendix A.

6. In 1964, I graduated with an A.B. (cum laude) from the University of Pennsylvania in Philadelphia, Pennsylvania. In 1968, I earned my M.D. from the University of Pennsylvania School of Medicine. From 1969 until 1972, I served as Research Associate with the rank of Lt. Commander in the U.S. Public Health Service at the National Institutes of Health, National Heart and Lung Institute, Laboratory of Biochemistry, Section on Enzymes, Bethesda, Maryland. From 1972 until 1974, I completed my residency in Neurology at UCSF.

7. From 1974 until 1980, I served as Assistant Professor of Neurology at UCSF. Between 1976 and 1988 I was a lecturer in the Department of Biochemistry and

Biophysics at UCSF. From 1979 until 1983, I was Assistant Professor of Virology in Residence at the University of California, Berkeley (UC Berkeley). In 1980 and 1981, I was Associate Professor of Neurology in Residence at UCSF. From 1981 until 1984, I was Associate Professor of Neurology at UCSF and from 1983 until 1984, Associate Professor of Virology in Residence at UC Berkeley. In 1984, I was appointed Professor of Neurology at UCSF and Professor of Virology in Residence at UC Berkeley. In 1988, I was appointed Professor of Biochemistry at UCSF.

8. In September 1972, two months after I began my residency at UCSF in the Department of Neurology, I admitted a female patient who was exhibiting progressive loss of memory and difficulty performing some routine tasks. I was surprised to learn that she was dying of a “slow virus” infection called Creutzfeldt-Jakob disease (CJD), which evoked no response from her body’s defenses. Next, I learned that scientists were unsure if a virus was really the cause of CJD since the causative infectious agent had some unusual properties. The amazing properties of the presumed causative “slow virus” captivated my imagination and I began to think that defining the molecular structure of this elusive agent might be an intriguing research project. The more that I read about CJD and the seemingly related diseases, such as kuru in the Fore people of New Guinea and scrapie in sheep, the more captivated I became.

9. My research is focused on infectious proteins called prions that cause fatal neurodegenerative diseases including BSE in cattle, scrapie in sheep, chronic wasting disease in deer and elk, and CJD in humans. Nascent prions are created by (1) spontaneous (or sporadic) refolding of a host protein, (2) genetic mutation or (3) exposure to exogenous prion. In mammals, prions are composed of a modified form of the prion

protein (PrP) designated PrP^{Sc} (the superscript “Sc” is for scrapie which is the most well-studied prion disease). Like other infectious pathogens, prions multiply but they do not have a nucleic acid genome to direct the synthesis of their progeny. Rather, PrP^{Sc} (or prions), can induce the normal, cellular prion protein, designated PrP^C, to refold. In doing so, it becomes infectious. The mis-folded prion proteins (PrP^{Sc}) can build up, creating masses of protein that rupture cells and cause microscopic holes in the brain. The destruction is so severe that the brain begins to resemble a sponge and death is certain. Prion diseases of humans and animals are 100% fatal. No vaccines, antidotes or cures exist to treat these disorders.

10. Since this structural transition in the prion protein underlies both the replication of prions and the pathogenesis of Central Nervous System (CNS) degeneration, much of the effort in my laboratory is devoted to elucidating the molecular events responsible for this process. Indeed, prion diseases seem to be disorders of protein conformation.

11. As a result of the unusual nature of prion diseases (e.g., the very small exposure to prions that can cause disease; resistance to heat, irradiation, and chemicals that might destroy or modify nucleic acids; incubation in animals and humans for years without any symptoms), controlling prion diseases is very difficult. Policy makers need to take immediate and aggressive measures to minimize and mitigate the risk of prion transmission between animals and humans. In doing so, this will ensure the safety of our food supply.

12. Evidence from numerous scientific studies supports the conclusion that a variant of Creutzfeldt-Jakob disease (vCJD), can be caused by ingestion of BSE-infected beef and beef products. Approximately 170 cases of vCJD have been attributed to

consumption of BSE-tainted beef and beef products (occurring primarily in the United Kingdom (UK)). It is unknown how many other people worldwide are harboring prions that cause vCJD. Some scientists have projected the possibility that thousands of people may be infected with vCJD prions. Recent testing of tonsils and appendices removed from otherwise-healthy individuals in the UK argues that thousands of people may be harboring vCJD prions from the consumption of BSE-infected beef. It is important to note that there is evidence of two iatrogenic transmissions of vCJD prions from human to human, to date.

13. Many researchers believe that there is a “species barrier,” meaning that humans are less susceptible than cattle to the prions causing BSE and vCJD. Comparing known cases of vCJD to estimated exposures to BSE-contaminated meat is not sufficient to establish that there is a species barrier for several reasons: First, the incubation period for prion diseases in humans typically is long (anywhere from two and up to as many as 40 years), thus there may be many individuals with vCJD prions who are currently asymptomatic. Second, many scientists are concerned that vCJD is under-diagnosed, given the unusual nature of the pathogen, the difficulty of an accurate diagnosis short of an autopsy, and that prion science is a relatively new field of study. Finally, the denominator in any estimate of a species barrier – the amount of infected tissue to which humans were exposed – can only be an approximation, based on limited test data and many assumptions. Risk assessment and risk management decisions about the safety of our food supply should be based on the concept that one infectious unit (or dose) of prions is sufficient to cause vCJD in humans.

14. In vCJD, prions accumulate not just in the brain and spinal cord but also in the lymphoid tissues, such as the tonsils and appendix, suggesting that prions enter the bloodstream. Sheep and rodent studies have shown that prions can be transmitted to healthy animals through blood transfusions from infected animals. Two recent cases in the UK argue that vCJD prions were transmitted between humans through blood transfusions. Because there is no reason to believe that BSE prions in cattle behave differently from those in other mammals, feeding bovine blood to cattle presents a risk of transmission of BSE prions. Therefore, a program to minimize the propagation of BSE prions should include, but not be limited to, a ban on feeding ruminant blood to other ruminants and to animals, a practice which I understand has been advocated by the U.S. Food and Drug Administration.

15. Given the evidence that prions are found in blood, prions may be present in any organ including muscle, since all tissues are perfused with blood. Patrick Bosque (now at the University of Colorado's Health Sciences Center) and I found prions in the hind limb muscles of mice at a level approximately 100,000-fold higher than that found in blood. Michael Beekes and his colleagues at the Robert Koch Institute in Berlin discovered high-levels of prions in virtually all skeletal muscles, not just in the hind limbs, after prions were fed to hamsters; and other scientists have found prions in the tongues of infected hamsters. Sheep infected with scrapie prions were found to have prions in both the hind and fore limb muscles. Similar findings have been observed in humans with CJD. My UCSF colleagues Jiri Safar and Stephen DeArmond found prions in the muscles of CJD patients, and Adriano Aguzzi and his colleagues at the University of Zürich identified prions in the muscles of 25% of the CJD patients they examined.

16. Based on these and other scientific studies, it is reasonable to contend that the consumption of beef and beef products from cattle harboring BSE prions presents a risk for humans. Regardless of whether the tonsils and distal ileum have been removed from cattle – and in the case of cattle 30 months of age and older, the brain, eyes, spinal cord, and trigeminal ganglia as well – these measures are unlikely to be sufficient to ensure the safety of the meat we consume. The only reliable way to minimize the risk of humans developing vCJD from BSE-infected cattle is to eliminate BSE-infected cattle from the food chain. And the only way to reduce the number of BSE-infected cattle entering the food chain is to require blanket testing of all slaughtered cattle so that animals testing positive for prions will be removed from the food supply.

17. Since the mid-1980s, scientists at my laboratory and around the world have attempted to develop methods for identifying prions that can serve as an alternative to the immunohistochemistry test (IHC). The IHC test can be unreliable, cumbersome and extremely time-consuming. For example in a recent publication by my colleagues Safar and DeArmond, IHC consistently detected prions in only 4 out of 18 regions (22%) in human CJD brains whereas the CDI (Conformation-Dependent Immunoassay) test developed at UCSF detected prions in all 18 regions (100%). IHC is routinely used by the USDA as their confirmatory test and is considered their “gold standard.” More streamlined and sensitive methods have been developed to conduct broader and more accurate testing for prions including the CDI. A significant portion of my career has been dedicated to the development and evaluation of sophisticated techniques for the detection of prions and subsequent diagnosis of BSE, scrapie, sporadic CJD and vCJD. A company

I founded, InPro Biotechnology, offers several sensitive and reliable prion tests which are being made available commercially.

18. It has been confirmed that two Japanese cows were diagnosed with BSE at 21 and 23 months of age in the fall of 2003. These cases help to demonstrate the necessity of blanket testing of all cattle at slaughter, including the testing of cattle less than 30 months of age. Neither of these two Japanese cows showed clinical signs of neurological dysfunction, thus neither would have been selected as a “high-risk” animal and designated for testing under current USDA protocols. These cows were only identified as positive for BSE prions because of the Japanese government's decision to adopt a policy of blanket testing for BSE at slaughter.

19. Experience in Europe has shown that, when countries have switched from only testing cattle showing clinical signs of neurological disorders to testing all animals above a certain age, the number of reported BSE cases increases. The European Union (EU) adopted regulations in 2001 requiring BSE screening of all animals over 30 months of age intended for the human food supply. (France, Germany, Italy and Slovenia choose to test all cattle that are over 24 months of age.) A May 2004 report from the EU Health and Consumer Protection Directorate-General shows that, as a result of testing all cattle slaughtered for human consumption above a specific age, more cases of BSE have been identified through this “active” testing than were identified through the testing of cattle that were determined by farmers or veterinary practitioners to exhibit clinical signs for BSE (referred to as “passive” testing). This active testing in the EU has shown that BSE-infected cattle may display no signs even though they harbor substantial numbers of prions that can be identified using a rapid test for BSE. Thus, active testing of cattle

slaughtered for human consumption has prevented BSE-infected cattle from entering the food chain. The rapid BSE tests that are currently available, as well as those that are becoming available, are sensitive enough to make universal screening for BSE practical, without imposing excessive costs.

20. Current scientific knowledge demonstrates the ability to detect prions in cattle less than 30 months of age: BSE prions were detected in two cows 21 and 23 months of age in Japan. In the UK, 84 cases of BSE have been found in cattle 30 months of age and younger. Two positive animals under 30 months of age have also been detected in Germany. Recent advances in BSE diagnostics including tests developed in my laboratory at UCSF are the most likely to detect low-levels of prion infectivity. In order to realize the human-health benefit of testing it will be important to utilize the most sensitive and specific detection methods available. We should not underestimate the value of removing all apparently healthy cattle with detectable levels of BSE prions from the food chain. Such animals do not exhibit clinical signs and thus, would not otherwise be excluded from the food supply. I believe that testing all slaughtered animals is the only rational policy for adequately protecting the human food supply.

21. Given that no country other than Japan routinely screens cattle of all ages entering the food supply, and that only France, Germany, Italy, and Slovenia require routine BSE testing of cattle beginning at 24 months of age, there is little empirical data on BSE in younger cattle. Because the amount of infected tissue necessary to cause vCJD in humans is not known, and because the distribution of prions in infected cattle over time is not completely understood, it is neither safe nor scientifically justified to assume that all

cattle under 30 months of age present no risk of harboring BSE prions and thus, cannot cause vCJD in humans.

22. Prion diseases are invariably fatal and no vaccine, antidote or cure exists to treat them. Similar to the human prion diseases, it is likely that the spontaneous (or sporadic) form of BSE accounts for many cases in cattle and thus prions will not disappear from livestock simply by eliminating prion-infected feeds. That being the case, it is imperative for the USDA to implement measures that address this public health threat and mitigate the risk of transmission as much as possible which should include the testing of all cattle slaughtered in the United States.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct. Executed on June 28, 2005.


Stanley B. Prusiner, M.D.