TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

IN THE UNITED STATES

Transmissible Spongiform Encephalopathies in the United States

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Cover
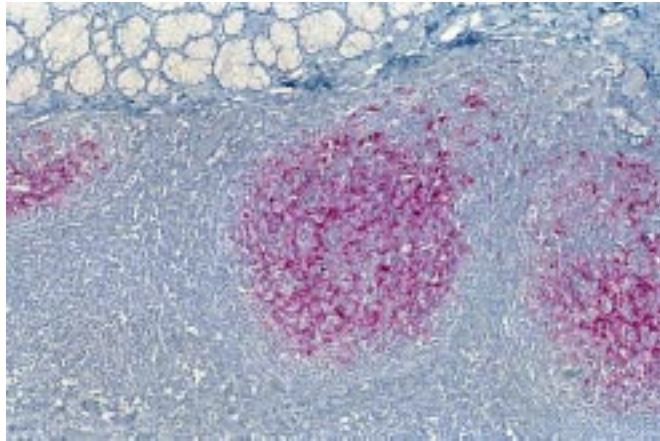
Photomicrograph of lymphoid follicles of the tonsil of a mule deer (*Odocoileus hemionus*) with chronic wasting disease (CWD). The dark red staining is the prion that is associated with chronic wasting disease. Immunohistochemical stain with monoclonal antibody 160.1.5. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

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Foreword

Following a recommendation by the CAST National Concerns Committee, the CAST Board of Directors authorized preparation of a report on transmissible spongiform encephalopathies in the United States.

Dr. William D. Hueston, Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park, and James L. Voss, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, served as cochairs for the report. A highly qualified group of scientists served as task force members and reviewers and participated in the writing and review of the document. They include individuals with expertise in human and animal food and feed, immunology and diagnostics, infectious diseases of humans and animals, medical microbiology, human and veterinary medicine, and virology.

The task force prepared an initial draft of the report, which was reviewed by the credited reviewers. The task force revised all subsequent drafts and the task force and credited reviewers reviewed the proofs. The CAST Executive and Editorial and Publications committees reviewed the document. The CAST staff provided editorial and structural suggestions and published the report. The authors are responsible for the report's scientific content.

On behalf of CAST, we thank the cochairs, authors, and reviewers who gave of their time and expertise to prepare this report as a contribution by the scientific community to public understanding of the issue. We also thank the employers of the scientists, who made the time of these individuals available at no cost to CAST. CAST recognizes and appreciates the financial support of the U.S. Department of Agriculture/Agricultural Research Service to partially assist in the development and completion of this report. CAST thanks all members who made additional contributions to assist in the preparation of this document. The members of CAST deserve special recognition because the unrestricted contributions they have made in support of CAST also have financed the preparation and publication of this report.

This report is being distributed widely including to members of Congress, the White House, the U.S. Department of Agriculture, the Congressional Research Service, the Food and Drug Administration, the Environmental Protection Agency, the Agency for International Development, the Office of Science and Technology Policy, and the Office of Management and Budget, and to media personnel and institutional members of CAST. Individual members of CAST may receive a complimentary copy upon request for a $3.00 postage and handling fee. The report may be reproduced in its entirety without permission. If copied in any manner, credit to the authors and to CAST would be appreciated.

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Interpretive Summary

The British government's March 20, 1996 announcement of a potential link between bovine spongiform encephalopathy (BSE), commonly called "mad cow" disease, and a new human illness, new variant Creutzfeldt-Jakob disease (nvCJD), focused attention on a unique group of animal and human diseases, the transmissible spongiform encephalopathies (TSEs).

Transmissible spongiform encephalopathies are fatal, neurodegenerative disorders that affect both animals and humans. The major animal forms of these diseases are BSE, scrapie (sheep and goats), chronic wasting disease (CWD) (deer and elk), transmissible mink encephalopathy (TME), and feline spongiform encephalopathy (FSE) (the expression of BSE in domestic cats). The human forms of these diseases are Creutzfeldt-Jakob disease (CJD), nvCJD whose causative agent is indistinguishable from BSE, Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), and Kuru.

Biology

The unusual properties of the agent responsible for the TSEs, along with the clinical and pathological features of these diseases, have led to speculation on the nature of the infectious agent. Three theories have withstood the test of time: (1) the virus hypothesis, (2) the virino hypothesis, and (3) the modified host protein or prion hypothesis. Although each theory is possible, none has been indisputably accepted.

Diagnostics

Distinctive neuropathology and the presence of a unique protease-resistant protein characterize all TSEs. Histopathology and immunohistochemistry of frozen or formalin-fixed tissues are the most commonly used diagnostics in most medical and veterinary diagnostic laboratories. Research is ongoing for other diagnostic tests that could be used antemortem.

Transmissible Spongiform Encephalopathies in Animals

Bovine spongiform encephalopathy was first described in Great Britain in November 1986 and has been diagnosed in nine other European countries — Belgium, Denmark, France, Liechtenstein, Luxembourg, the Netherlands, Portugal, the Republic of Ireland, and Switzerland. BSE is a nervous disorder that is expressed in adult cattle between 2 and 8 years of age after a long incubation period and is always fatal. Though maternal transmission from cow to calf is thought to occur, the main route of transmission during the United Kingdom BSE epidemic was through feeding contaminated ruminant- derived protein. The origin of the BSE agent is unexplained. Novel TSEs have occurred in exotic ruminants, exotic felids, and domestic cats, all of which are indistinguishable from BSE on strain-typing of the agent.

Bovine spongiform encephalopathy has never been detected in the United States. In May 1990, the United States began an aggressive BSE surveillance program to ensure timely detection and swift response in the unlikely event that an introduction were to occur. In 1989, the United States prohibited the importation of ruminants and most ruminant products from countries affected with BSE. The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has conducted a trace-back effort to locate each of the imported cattle originating from BSE countries between 1980 and the ban. No evidence of BSE has been found in any of these imported animals. On December 12, 1997, APHIS prohibited importation of live ruminants and most ruminant products from the rest of Europe, pending a thorough risk assessment.

Scrapie is a fatal, degenerative disease affecting the central nervous system of sheep and goats. First recognized as a disease of sheep in Great Britain and other countries of western Europe more than 250 years ago, scrapie has been reported in most sheep-raising countries, with a few notable exceptions such

as New Zealand and Australia. Signs or effects of the disease usually do not appear until 2 to 5 years after the animal is infected. Sheep may live 1 to 6 months or longer after the onset of clinical signs, but death is inevitable. It is generally accepted that scrapie is an infectious, contagious disease in which genetics plays an influential role. There is no scientific evidence that scrapie poses a risk to human health.

The first case of scrapie was diagnosed in the United States in 1947. This disease was in a sheep of British origin imported through Canada. Through August 1, 1999, scrapie had been confirmed in 950 flocks since its introduction in the late 1940s. Various forms of control and/or eradication programs have existed since 1952. A Scrapie Flock Certification Program and regulations to restrict the interstate movement of high-risk sheep from known infected and source flocks was begun in the early 1990s. A Certification Program update effective September 1999 strengthened control measures.

Chronic wasting disease was first recognized in the United States in 1967. It naturally affects wild and captive mule deer, white-tailed deer, and Rocky Mountain elk in limited areas in southeastern Wyoming and north central Colorado in the United States and was recently diagnosed among privately owned elk on game farms. CWD's mode of transmission is unknown, but epidemiologic evidence strongly suggests that lateral transmission occurs among deer and elk. There is no evidence that CWD is a foodborne disease associated with consumption of animal protein. Surveillance of free-ranging deer and elk has been ongoing since 1983. As of December 1999, CWD has been detected in captive elk herds in South Dakota, Nebraska, Montana, Colorado, Oklahoma, and Saskatchewan, Canada. Surveys of brains from deer and elk in surrounding and other states have detected no evidence of CWD. Although CWD shares many clinical and pathological characteristics with the other TSEs such as scrapie and BSE, strain typing of tissues from a mule deer with CWD indicates that it may be a separate disease entity and not caused by the same strain of agent as BSE or scrapie.

Transmissible mink encephalopathy was first identified in Wisconsin in the United States in 1947 and has since been observed in ranch-raised mink in Canada, Finland, Germany, and the former Soviet Union. Similar to other TSEs, TME is characterized by the insidious onset of progressive neurological disease. The origin of the transmissible agent in TME seems to be contaminated feedstuffs. To date, five

U.S. outbreaks of TSE have been recorded: in 1947, 1961, 1963 (two outbreaks), and 1985. Research and surveillance continue at the University of Wisconsin.

Information about **feline spongiform encephalopathy** and BSE-associated TSEs of exotic ruminants and members of the cat family have been disseminated to accredited veterinarians throughout the United States to increase their awareness of the condition. No cases of FSE or TSEs of exotic animals have been detected in the United States.

Transmissible Spongiform Encephalopathies in Humans

The most common form of TSE in humans is **Creutzfeldt-Jakob disease**, which occurs with an annual incidence of approximately one case per million population worldwide. CJD is rapidly progressive and invariably fatal and occurs in three disease forms, usually affecting people between the ages of 55 and 75 years: sporadic (about 85%), familial (5 to 15%), or iatrogenic (medically induced) (rare) associated with use of contaminated medical products or devices.

Gerstmann-Sträussler-Scheinker syndrome is a familial form of CJD that is much less common. It occurs in association with various specific genetic mutations at different sites of the prion protein gene. Familial clusters have been reported in North America, Europe, Israel, and Japan.

Fatal familial insomnia is an inherited TSE that shares a similar prion protein gene mutation with familial CJD. However, patients have a distinct clinical manifestation. FFI has been reported in families from Australia, Austria, Britain, France, Germany, Italy, Japan, and the United States. A sporadic form of FFI has been described recently.

New variant Creutzfeldt-Jakob disease was identified in 1996 when 10 unusually young patients in the United Kingdom developed clinical features atypical of CJD and an apparently unique brain pathologic profile. The patients' age of onset ranged from 16 to 39 years. The subsequent occurrence of additional nvCJD cases in the United Kingdom and accumulating laboratory evidence strongly support the conclusion that nvCJD resulted from the transmission to humans of the agent causing BSE. As of September 4, 2000, 82 definite and probable cases of nvCJD have been officially

reported in the United Kingdom, three in France, and one in the Republic of Ireland.

Kuru is primarily of historical importance now. Kuru, a TSE restricted to the Fore people of New Guinea, was spread from person-to-person through ritualistic cannibalism (now outlawed).

Relationship between TSEs in Animals and Humans

Most TSEs are species-specific, with no evidence of natural transmission between animals and humans. In contrast, strong epidemiologic and laboratory evidence indicates that BSE may have been transmitted to humans causing nvCJD. Although the precise mode of transmission of the BSE agent has not been described, dietary exposure through consumption of contaminated animal products seems most likely. Experimental studies to date have increased the strength of evidence that the agents causing nvCJD and BSE are indistinguishable.

Summary

Introduction

The British government's March 20, 1996 announcement of a potential link between bovine spongiform encephalopathy (BSE), commonly called "mad cow" disease, and a new human illness, new variant Creutzfeldt-Jakob disease (nvCJD), focused attention on a unique group of animal and human diseases, the transmissible spongiform encephalopathies (TSEs).

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Diagnostics

Distinctive neuropathology and the presence of a unique protease-resistant protein (PrP) characterize all TSEs. Histopathology and immunohistochemistry of frozen or formalin-fixed tissues are the most commonly used diagnostics in most medical and veterinary diagnostic laboratories. Immunoblot and western blot are used primarily in research facilities. Research is ongoing for other diagnostic tests that could be used antemortem.

Transmissible Spongiform Encephalopathies in Animals

Bovine spongiform encephalopathy was first described in Great Britain in November 1986 (Wells et al. 1987). The disease now has been diagnosed in nine other European countries — Belgium, Denmark, France, Liechtenstein, Luxembourg, the Netherlands, Portugal, the Republic of Ireland, and Switzerland. BSE is a nervous disorder that is expressed in adult cattle between 2 and 8 years of age after a long incubation period. The clinical course of the disease is progressive and always fatal. Though maternal transmission from cow to calf is thought to occur, the main route of transmission during the United Kingdom (U.K.) BSE epidemic was through feeding contaminated ruminant-derived protein. The origin of the BSE agent is unexplained. Two major theories have been proposed: (1) the adaptation of the scrapie agent to a strain transmissible from sheep or goats to cattle or (2) that BSE has existed in cattle populations for a long time in an unrecognized or clinically silent form. The emergence of the epidemic under either hypothesis is linked to enhanced survival of the agent in ruminant-derived protein subsequent to changes in the rendering process. The enhanced survival of the agent allowed sufficient exposure so that cattle were infected and developed the disease within their life spans. The epidemic was amplified by the recycling of infected cattle parts through rendering and subsequent feeding of ruminant-derived protein. Novel TSEs have occurred in exotic ruminants, exotic felids, and domestic cats, all of which are indistinguishable from BSE on strain-typing of the agent.

Scrapie is a fatal, degenerative disease affecting the central nervous system of sheep and goats. First recognized as a disease of sheep in Great Britain and other countries of western Europe more than 250 years ago, scrapie has been reported in most sheep-raising countries, with a few notable exceptions such as New Zealand and Australia (Parry 1983). Signs or effects of the disease usually do not appear until 2 to 5 years after the animal is infected. Sheep may live 1 to 6 months or longer after the onset of clinical signs,

but death is inevitable. It is generally accepted that scrapie is an infectious, contagious disease in which genetics plays an influential role. The means of natural transmission have not been fully defined, but it is thought to be spread most commonly from ewe to offspring and to other lambs in contemporary lambing groups through contact with the placenta and placental fluids. There is no scientific evidence that scrapie poses a risk to human health. Preventive steps are addressed in this report.

Chronic wasting disease naturally affects wild and captive mule deer, white-tailed deer, and Rocky Mountain elk in limited areas in southeastern Wyoming and north central Colorado in the United States and was recently diagnosed among privately owned elk on game farms in Saskatchewan, South Dakota, Montana, Nebraska, Oklahoma, and Colorado. CWD's mode of transmission is unknown, but epidemiologic evidence strongly suggests that lateral transmission occurs among deer and elk. There is no evidence that CWD is a foodborne disease associated with consumption of animal protein. To prevent the geographic spread of CWD, free-ranging deer and elk are not transplanted or moved from the endemic areas of Wyoming and Colorado. Guidelines are being developed for the elk-farming industry, and the various sectors of the public are being targeted for educational offerings.

Transmissible mink encephalopathy was first identified in Wisconsin in the United States in 1947 and has since been observed in ranch-raised mink in Canada, Finland, Germany, and the former Soviet Union. Similar to other TSEs, TME is characterized by the insidious onset of progressive neurological disease. The origin of the transmissible agent in TME seems to be contaminated feedstuffs.

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Transmissible Spongiform Encephalopathies in the United States

Bovine spongiform encephalopathy has never been detected in the United States, and the Ani-

mal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) and other government agencies have taken a series of preventive measures and implemented an aggressive surveillance system (U.S. Department of Agriculture 1999a) to prevent its occurrence. In 1989, the United States prohibited the importation of ruminants and most ruminant products from countries affected with BSE. APHIS has conducted a trace-back effort to locate each of the imported cattle originating from BSE countries between 1980 and the ban. No evidence of BSE has been found in any of these imported animals. On December 12, 1997, APHIS prohibited importation of live ruminants and most ruminant products from the rest of Europe, pending a thorough risk assessment. In May 1990, the United States began an aggressive and active BSE surveillance program to ensure timely detection and swift response in the unlikely event that an introduction were to occur. BSE is a notifiable disease as specified in Title 9 Code of Federal Regulations (CFR), Parts 71 and 161. More than 250 federal and state regulatory veterinarians are specially trained to diagnose foreign animal diseases, including BSE. APHIS is the lead agency in the surveillance program, which also includes assistance from the Food Safety Inspection Service (FSIS).

The initial risk analyses done by APHIS in 1991 described significant differences in feeding and management practices between the United States and the United Kingdom that are believed to be important in decreasing the risk of BSE. A U.S. Food and Drug Administration (FDA) regulation that prohibits feeding most mammalian protein to ruminants went into effect August 4, 1997 (Title 21, CFR, Part 589.2000). These extra measures were taken to preclude the remote possibility of entry of BSE into the United States, and to prevent its recycling if the disease should occur.

The first case of **scrapie** was diagnosed in the United States in 1947. This disease was in a sheep of British origin imported through Canada. Through August 1, 1999, scrapie had been confirmed in 950 flocks since its introduction in the late 1940s. Various forms of control and/or eradication programs have existed since 1952. A Scrapie Flock Certification Program and regulations to restrict the interstate movement of high-risk sheep from known infected and source flocks was begun in the early 1990s. In 1997, the sheep industry leaders thought there was a need for further regulations to strengthen current scrapie control measures. They asked APHIS to publish an Advanced Notice of Public Rulemaking (ANPR) to solicit comments on the best approach for scrapie control. The ANPR was published in January 1998 and eventually led to an update of the Certification Program effective July 1999 (U.S. Department of Agriculture 1999b). Rules on interstate movement of sheep and goats as well as on pilot projects to evaluate flock cleanup plans based on testing have been proposed (U.S. Department of Agriculture 2000a). The proposed rule was published in November 1999.

Chronic wasting disease was first recognized in captive mule deer housed at a Colorado research facility in 1967. A similar condition was diagnosed in captive deer at a Wyoming research facility in 1978 and confirmed in Rocky Mountain elk. In addition to the cases at the research facilities, CWD primarily has been confined to free-ranging deer and elk in a 10-county endemic area in north central Colorado and southeastern Wyoming. As of December 1999, CWD has been detected in captive elk herds in South Dakota, Nebraska, Montana, Colorado, Oklahoma, and Saskatchewan, Canada. Although CWD shares many clinical and pathological characteristics with the other TSEs such as scrapie and BSE, strain typing of tissues from a mule deer with CWD indicates that it may be a separate disease entity and not caused by the same strain of agent as BSE or scrapie (Bruce et al. 1997). Surveillance for CWD in free-ranging deer and elk in Colorado and Wyoming has been ongoing since 1983. Surveys of brains from deer and elk in surrounding and other states have detected no evidence of CWD.

Transmissible mink encephalopathy was first diagnosed in the United States in 1947. To date, five U.S. outbreaks have been recorded: in 1947, 1961, 1963 (two outbreaks), and 1985. Research and surveillance continue at the University of Wisconsin.

Information about **feline spongiform encephalopathy** and BSE-associated TSEs of exotic ruminants and felids have been disseminated to accredited veterinarians throughout the United States to increase their awareness of the condition. No cases of FSE or TSEs of exotic animals have been detected in the United States.

1   Introduction

William D. Hueston and James L. Voss

Transmissible spongiform encephalopathies have been diagnosed in mammals including humans worldwide. These fatal degenerative neurologic diseases are associated with a unique agent called prions (proteinaceous infectious particles); however, other infectious agents also have been incriminated. The diseases are difficult to research because of the causative agents' long incubation period and the difficulty in developing an antemortem diagnostic test.

The major animal forms of TSE diseases are bovine spongiform encephalopathy, scrapie (sheep and goats), chronic wasting disease (deer and elk), transmissible mink encephalopathy, and feline spongiform encephalopathy (the expression of BSE in domestic cats). The human forms of these diseases are Creutzfeldt-Jakob disease, a variant of CJD whose causative agent is indistinguishable from BSE, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, and Kuru.

In the United Kingdom, BSE, tagged "mad cow disease" by the British press, resulted in severe death losses of cattle and a probable link to a new human disease, nvCJD. The outbreak of BSE has been associated with feeding animal protein to cattle. The occurrence of BSE and nvCJD served to mobilize U.S. federal agencies to prevent the disease from spreading to the United States. No natural cases of BSE or nvCJD have been reported in the United States.

Several forms of animal TSEs occur in the United States: scrapie, naturally occurring in sheep and goats; CWD, occurring in deer and elk; TME; CJD (not the variant form); GSS; and FFI. Sheep scrapie, first diagnosed in the United States in 1947, seems to be widely dispersed but infrequent. In mink, TME occurs sporadically with only five outbreaks from 1947 to 1985. Currently, CWD is restricted to wild populations of deer and elk in an isolated region of the western United States and on several farmed elk herds in five states in the United States and in one provinces in Canada. The emergence of the BSE epidemic in Great Britain and the staggering cost of its control have energized further TSE-related research and regulatory action in the United States and around the world.

Nobel prizes have been awarded to two U.S. scientists for their research on TSEs: D. C. Gadjusek for his work with Kuru and S. B. Pruisner who pioneered the concept of "prions" (proteinaceous infectious particles) as the causative agent of TSEs. The validity of the prion theory is still debated, and TSE diseases of animals and humans continue to grab headlines in both the lay and scientific press.

This paper seeks to provide a comprehensive background on TSE diseases in animals. The first section will explore the nature of the causative agent. Subsequent sections will review the challenge of diagnosing each of the known TSEs of animals. The relationship between BSE and human disease will be discussed, along with an overview of other human TSEs. The paper will conclude with an overview of the status of TSEs in animals in the United States.

2 Biology of Transmissible Spongiform Encephalopathies

Richard Rubenstein

Introduction

Following the successful transmission of sheep scrapie to laboratory animals (Chandler 1961; Kimberlin and Walker 1977; Marsh and Kimberlin 1975), studies were performed that led to the discovery of a protease-resistant protein, termed PrP27-30, that copurified with infectivity and had an apparent molecular mass of 27 to 30 kilodaltons (kDa) (Bolton, McKinley, and Prusiner 1982; Prusiner et al. 1982). The amount of this protein, which was found only in infected tissue and not in control samples, correlated with the levels of infectivity (Hope and Manson 1991). This form of the prion protein was subsequently found to be a partial digestion product of the infection-induced modified host glycoprotein, PrP^{Sc} or PrP^{res}.

The unusual properties of the infectious agent responsible for the TSEs, along with the clinical and pathological features of these diseases, have led to speculation on the nature of the infectious agent. Of all the theories proposed, three have withstood the test of time: (1) the virus hypothesis, (2) the virino hypothesis, and (3) the modified host protein or prion hypothesis. Proponents of the virus hypothesis believe that the infectious agent is a typical virus (containing a virus-specific nucleic acid and viral-coded protein) with unusual properties. The virino hypothesis suggests that the infectious agent is a small, noncoding nucleic acid that is surrounded and protected by a host-coded protein. The prion hypothesis states that the infectious agent is composed exclusively of a post-translationally modified host protein.

Supporters of any of these theories must be able to explain the unusual physical-chemical characteristics of the infectious agent, e.g., resistance to inactivation, small size, failure of the infected host to mount an immune response, and failure to observe virus particles in infected tissue by electron microscopy. A major hurdle for any of the theories is to explain the existence of different scrapie strains. Even within a genetically controlled host background, scrapie strains differ in their biological and pathological properties. Although each theory is possible, none has been indisputably accepted. See Appendix C for more details for each theory.

3 Transmissible Spongiform Encephalopathy Diagnostics

Janice M. Miller

Availability of methods to diagnose TSEs has changed markedly in the last two decades. Prior to that time, a preliminary diagnosis usually was based on the observation of typical clinical signs, with final confirmation provided by histopathologic examination of the brain. Lesions were not detectable by gross visual inspection; however, light microscopic examination of tissue sections revealed a characteristic "spongy" appearance that was produced by degenerative changes in specific regions of the brain. Another diagnostic tool, available in a few research laboratories, depended on the detection of "scrapie-associated fibrils" (SAF) in diseased brain by using electron microscopy. The most stringent and convincing diagnostic test for TSE was based on animal inoculations, which were necessary to provide proof of disease transmissibility. The expense and time-consuming nature of this procedure, however, limited its use almost exclusively to research, rather than diagnostic, purposes.

In the early 1980s, the researchers identified PrP^{Sc} as a potential marker that could be used to develop specific and economical diagnostic tests. Initially, successful attainment of this goal seemed unlikely when PrP was found in normal as well as in TSE-affected brain tissue. Furthermore, because animals (and people) with TSEs did not show an immune response, it was not possible to develop tests to detect PrP-specific antibody. Fortunately, scientists found that such antibodies could be generated experimentally through repeated exposure of an animal from one species to highly concentrated and purified PrP^{Sc} from another species. These antibodies then would detect PrP in tissues.

The first use of PrP antibodies to diagnose a TSE was with an immunoblot test. For this procedure, PrP is extracted from tissue and incubated with a proteolytic enzyme. The enzymatic digestion process destroys normal PrP (PrP^c or PrP^{sen}), whereas the conformationally abnormal PrP (PrP^{Sc} or PrP^{res}) found in TSE cases remains. Following digestion, the tissue extract is purified, concentrated, attached to a membrane, and exposed to PrP antibody. If the test sample contains abnormal PrP, an irreversible anti-gen-antibody reaction occurs. The product of this reaction then can be detected by routine immunologic procedures.

Western blot, a modification of the immunoblot method, more commonly is used for TSE diagnosis because of its more reliable specificity. In this technique, the tissue extract is placed in a gel and an electric current is passed through it. The current causes any proteins present to migrate at different rates, depending on their size. The proteins then are transferred from the gel to a membrane, and a standard immunoblot-type procedure is performed. Because the size range of PrP molecules is limited, specificity of the antigen-antibody reaction product can be assessed by comparing its position on the membrane to that of protein markers with known molecular weights.

The primary disadvantage of immunoblot and western blot techniques for TSE diagnosis is that the PrP extraction process is fairly laborious, requiring special equipment and technical expertise usually found only in some research laboratories. Several groups are working to simplify the PrP extraction procedure and to devise alternative detection methods that would be more suitable for diagnostic situations. The success of these approaches is especially critical for future development of rapid tests that can be automated. Such systems would be required for large-scale TSE surveillance of tissue, blood, and biological or pharmaceutical products.

The second major advance in TSE diagnosis has come from application of a method known as immunohistochemistry (IHC) (Figures 3.1–3.2). This procedure has been used for many years by diagnostic pathologists for light microscopic identification of various cell proteins, e.g., tumor cell markers or infectious agents in tissues. Similar to the immunoblot and western blot methods, IHC relies on identifying a protein antigen by using specific antibody. For IHC, however, it is not necessary to prepare a tissue extract because the test is conducted on very thin tissue slices. Although the test can be performed on fresh (frozen) tissue, the most common practice is to use tissues that have been fixed in formalin and em-
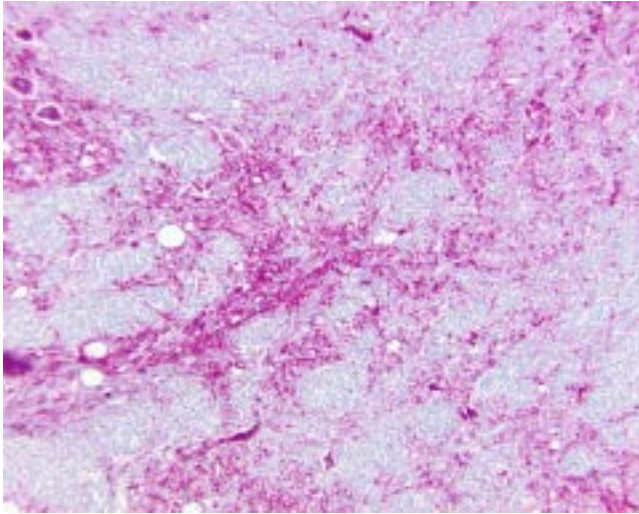
Figure 3.1. Nucleus of the spinal tract of the trigeminal nerve in the medulla oblongata of a sheep with scrapie. The red staining indicates prion protein deposition. Immunohistochemical stain with monoclonal antibodies F89/160.1.5 and F99/97.6.1. Photograph courtesy of Dr. Daniel H. Gould, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins.
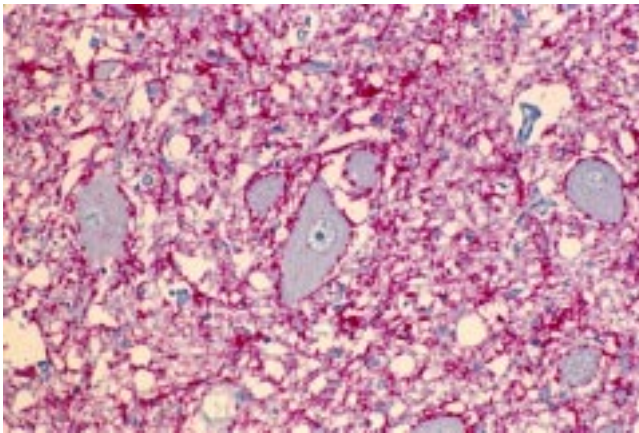

Figure 3.2. Photomicrograph of the dorsal motor nucleus of the vagus nerve of a mule deer (*Odocoileus hemionus*) with chronic wasting disease. The dark red staining is the prion that is associated with chronic wasting disease. Immunohistochemical stain with monoclonal antibody 160.1.5. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

bedded in paraffin, a process routinely used to prepare tissues for light microscopic examination by a pathologist. Because IHC is a common procedure in most medical and veterinary diagnostic laboratories, only minimal modifications are required to adapt the method for use in TSE diagnosis. The most unique requirement is that the tissue slices must be heated

to a high temperature, e.g., autoclaved or exposed to microwaves or formic acid, to expose PrP antigenic sites that otherwise are not available for reaction with antibody because of the effects of formalin fixation. Selecting an appropriate antibody is also very important because some of the antibodies used for immunoblot and western blot detection of PrP do not react with the antigenic sites recognized in IHC. Furthermore, whereas some antibodies detect PrP from many different animal species, others are quite species specific (this situation also occurs with immunoblot procedures). The main disadvantage of IHC is the difficulty in developing procedures suitable for rapid screening of many samples and subjectivity of tests. Furthermore, IHC also can miss detecting PrP^{Sc} early in the pathogenesis of TSEs. Nevertheless, because of its simplicity and relatively low cost, IHC is likely to be the most commonly used TSE diagnostic test for at least several years.

Because brain biopsy of living patients is a potentially dangerous surgical procedure, TSE diagnostic tests are applied primarily to tissues obtained after death. In some TSEs, the abnormal form of PrP is found in lymphoid tissues as well as in brain. Several recent studies have suggested that these tissues may be useful sites for antemortem testing by IHC. To date, this approach has been successfully demonstrated only in sheep scrapie and in one form of human TSE (nvCJD). Tissues being investigated include tonsil in humans and tonsil and lymphoid tissue from the third eyelid in sheep. The demonstration of PrP in these lymphoid tissues has led to speculation that circulating white blood cells also may contain the protein, which would allow development of a blood test. Even if this approach proves successful in scrapie and nvCJD, however, the method may not be universally applicable for TSE diagnosis because a blood-borne phase of PrP may not be characteristic of every form of the disease or for all potential host species.

Other diagnostic tests for TSE that do not rely on detection of PrP have been reported, but most of them are not absolutely specific. The most widely used is a test for a particular protein (14-3-3) that is released into the cerebrospinal fluid as a result of damage to brain cells. The procedure seems to work reasonably well in humans because other possible causes of neuronal damage can be ruled out and because suitable samples of cerebrospinal fluid are relatively easy to collect. It has been more difficult, however, to fulfill these criteria when dealing with nonhuman species; thus, it is unlikely that this test will be applied widely.

4 Transmissible Spongiform Encephalopathies in Animals

Bovine Spongiform Encephalopathy

William D. Hueston and Andrea Vicari

Introduction

Bovine spongiform encephalopathy was first described in Great Britain in November 1986. It is a nervous system disorder that affects adult cattle between 2 and 8 years of age after a long incubation period (time between exposure and clinical onset of disease). Affected cattle show changes in sensation (sensitivity to sound, light, and touch), mental abilities, and movement. The clinical course is progressive and always fatal. Because the clinical signs are not unique to BSE, an accurate diagnosis of BSE still depends on histopathological analyses of brain tissue taken after death of the affected cattle. Though maternal transmission (infection being passed from cow to calf) seems to occur (Wilesmith et al. 1997), the main route of transmission during the U.K. BSE epidemic was associated with feeding contaminated ruminant-derived protein (Wilesmith et al. 1988).

Origin

Epidemiological analyses in Great Britain found that the earliest suspected clinical cases occurred in April 1985. The only common exposure experienced by all the farms with BSE cases has been the incorporation of meat and bone meal, a rendered animal protein, into cattle feed. The exposure that resulted in the emergence of BSE in the United Kingdom seems to have begun in 1981–1982 (Wilesmith et al. 1988). Subsequent mathematical models that use estimates of incubation time and spread of the disease led other scientists to postulate that exposure already had started in the early 1970s (Anderson et al. 1996).

The origin of the BSE agent is still unexplained; two major theories have been proposed (Wilesmith et al. 1988). The first suggests adaptation of the scrapie agent to a strain transmissible from sheep or goats to cattle. The second hypothesis states that BSE has existed in cattle populations for a long time in an unrecognized or clinically silent form. The emergence of the epidemic under either hypothesis is linked to enhanced survival of the agent in ruminant-derived protein subsequent to changes in the rendering process. The enhanced survival of the agent allowed sufficient exposure so that cattle were infected and developed the disease within their life spans. The epidemic was amplified by the recycling of infected cattle tissues by rendered ruminant-derived protein in feed prior to the recognition of the disease's epidemiology and the implementation of effective control measures.

The recognition of the sporadic nature of classical human CJD, occurring in about one case per million people worldwide, led to a hypothesis that all TSE diseases may occur spontaneously. Extrapolating to cattle, the hypothesis suggests that BSE (or any other cattle TSE that might emerge in the future) may occur as a random, spontaneous, but rare event wherever cattle exist. Anecdotal evidence linking the rare outbreaks of TME to the practice of feeding nonambulatory (downer) cows to mink has been cited in support of this hypothesis (Marsh 1993). This epidemiological scenario, if true, would have important implications for countries currently considered BSE free. However, no evidence exists to prove the existence of spontaneous BSE.

Worldwide Incidence

As of May 2000, 176,792 confirmed BSE cases on 35,041 farms had been reported in the United Kingdom (U.K. Ministry of Agriculture, Fisheries, and Food 2000). Figure 4.1 reports the annual distribution of BSE cases for the years up to 1998 (International Office of Epizootics 1999). After its emergence in the mid-1980s, the number of BSE cases in Great Britain peaked in 1992. During that year, as many as 1,000 new cases were reported each week. The number of cases has gradually decreased, and the British BSE epidemic is expected to die out early in the twenty-first century. The most critical control measures seem to have been (1) mandatory notifica-
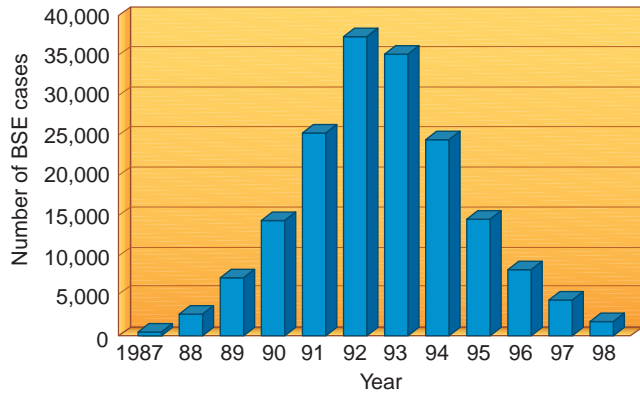
Figure 4.1.   Number of bovine spongiform encephalopathy cases reported in the United Kingdom, 1987-1998.

tion of suspect cases and consequent farmer compensation, (2) control of live animal movements, (3) efficient diagnostic capabilities, (4) prohibitions on feeding ruminant-derived rendered protein to all ruminants, and (5) stringent rules for rendering raw materials.

Although experiencing dramatically lower rates than in the United Kingdom, nine other European countries — Belgium, Denmark, France, Liechtenstein, Luxembourg, the Netherlands, Portugal, the Republic of Ireland, and Switzerland — have reported cases in native cattle. Table 4.1 displays the total number of cases for each country (International Office of Epizootics 1999). Epidemiological studies in some of these countries have shown that the likely cause of infection was exposure to feed containing BSE-contaminated protein products of animal origin, much of it originating from the United Kingdom although not necessarily imported through the most direct route (Hornlimann, Guidon, and Griot 1994). In addition, some cattle exported from Great Britain have succumbed to BSE in Canada, Denmark, the Falkland Islands, Ireland, Germany, the Sultanate of Oman, and Italy (International Office of Epizootics 1999).

Table 4.1.   Number of BSE cases in native cattle reported worldwide, 1987–1998 (International Office of Epizootics, 1999)

Country	Number of BSE cases
United Kingdom	175,590
Rep. of Ireland	347
Switzerland	283
Portugal	197
France	49
Belgium	7
Netherlands	4
Liechtenstein	2
Luxembourg	1

Epidemiological investigations suggest that large numbers of potentially exposed cattle and large amounts of presumably contaminated ruminant-derived protein were exported from Great Britain, principally to European countries. Consequently, more countries may be experiencing BSE than are currently recognized (Schreuder et al. 1997). In addition, the annual number of cases of BSE in several European countries continues to rise. Implementation of an effective control program across Europe has lagged behind the United Kingdom.

BSE also has been associated with the emergence of TSEs in exotic ruminants (e.g., nyala, gemsbok), exotic cats (e.g., cheetah, puma), and domestic cats (FSE). The agents isolated from these cases are indistinguishable from BSE; therefore, these diseases are considered to have resulted from exposure to BSE-contaminated feeds.

Conclusion

Several questions on the nature of the BSE agent and its epidemiology remain unanswered. The next few years should bring further knowledge on the pathogenesis of BSE, in particular, further data on infectivity distribution and concentration in tissues of affected cattle. This information should allow reassessment of the effectiveness of public health measures established to prevent human exposure to BSE. Live animal tests are likely to become available. As with methods of mass screening of carcasses, the efficacy of such tests will need to be carefully assessed under field conditions and on large populations. Furthermore, the sensitivity of the tests for identifying affected animals early in the pathogenesis of BSE must be evaluated.

In spite of incomplete understanding of the disease and the lack of live animal diagnostic tests, BSE can be prevented and controlled. The cardinal point in BSE control is the willingness of the cattle industry, veterinarians, renderers, and animal feed companies to implement and carry out measures such as disease surveillance and feed bans.

Scrapie

Linda A. Detwiler

Scrapie is a fatal, degenerative disease affecting the central nervous system of sheep and goats. First recognized as a disease of sheep in Great Britain and other countries of western Europe more than 250 years ago, scrapie has been reported in most sheep-

raising countries, with a few notable exceptions such as New Zealand and Australia (Parry 1983).

Clinical Signs

Signs of scrapie may vary widely among individual animals and breeds of sheep and are slowly progressive in their development. Signs or effects of the disease usually do not appear until 2 to 5 years after the animal is infected. Early signs include subtle changes in behavior or temperament. These changes are followed by other signs that may include tremor, especially of the head and neck; loss of coordination; scratching and rubbing against fixed objects; weight loss, despite retention of appetite; biting of feet and limbs; lip smacking; and gait abnormalities, including high stepping of the forelegs, hopping, and swaying of the back end.

An infected animal may appear normal at rest if left undisturbed. However, when stimulated by a sudden noise, excessive movement, or the stress of handling, the animal may tremble or fall in a convulsive-like state. Sheep may live 1 to 6 months or longer after the onset of clinical signs, but death is inevitable. Several other problems can cause clinical signs similar to scrapie in sheep, including ovine progressive pneumonia, listeriosis, and rabies; external parasites (lice, mites); pregnancy toxemia; and toxins.

Transmission

Over the years, the transmissibility of scrapie has been debated. Initially, arguments centered on whether the origin was genetic or infectious. Parry (1964) thought that scrapie was an autosomal recessive genetic disease that was not naturally infectious. He did concede that affected animals harbored a transmissible agent that was infectious by artificial routes.

Evidence of transmissibility of scrapie was proven when Cuille and Chelle (1936) successfully transmitted the disease from affected sheep to healthy ones by intra-ocular injection. Chandler (1961, 1962, 1963) added to this discovery by transmitting scrapie to mice. Later data indicated that scrapie was a naturally occurring contagious disease caused by an infectious agent (Brotherston et al. 1968; Dickinson et al. 1974; Hourrigan et al. 1979). Now, it is generally accepted that scrapie is an infectious, contagious disease in which genetics plays an influential role that is not completely understood. The means of natural transmission have not been defined fully.

The scrapie agent is thought to be spread most commonly from ewe to offspring and to other lambs in contemporary lambing groups through contact with the placenta and placental fluids (Onodera et al. 1993; Pattison et al. 1972, 1974; Race, Jenny, and Sutton 1998). Scrapie has not been shown to be transmitted via semen. However, 2 of 3 research projects using experimentally infected sheep suggest that embryos may play a role in the spread of scrapie (Foster et al. 1992, 1996). Certain aspects of all three embryo studies that have been questioned are being repeated.

Genetic variations among different breeds of sheep may play a role in whether sheep will become infected and how quickly clinical signs appear. Researchers in Edinburgh identified a gene called Sip (for scrapie incubation period) that controls the incubation period of scrapie in Cheviot and Swaledale sheep. Those individuals with "short" incubation alleles usually develop signs between 2 and 5 years of age. Sheep with "long" incubation alleles often die from what seem to be natural causes before the incubation period is complete. Because the incubation period can be longer than 5 years, it is not known to what extent or under what conditions infected sheep with the long incubation alleles might be able to transmit the disease to healthy sheep. It is likely that the PrP gene and the Sip gene are the same (Carlson et al. 1986; Hunter et al. 1987, 1989; Westaway et al. 1987). Further research involving additional breeds has suggested that genetic influence may extend beyond incubation length to conferring some degree of disease resistance (Belt et al. 1995; Clouscard et al. 1995; Hunter et al. 1993, 1994; Ikeda et al. 1995; Laplanche et al. 1993a, b; Westaway et al. 1994; O'Rourke, Melco, and Mickelson 1996).

Studies have found no scientific evidence that scrapie poses a risk to human health (Harries Jones et al. 1988; Kondo and Kuroiiwa 1982). Epidemiologic studies indicate no evidence that scrapie of sheep and goats is transmitted to humans through contact on the farm, at slaughterhouses, or in butcher shops (Brown et al. 1987).

Prevention and Control

Routine methods of preventing a laterally transmitted disease are vaccination, quarantine, with testing and removal and/or prohibition of animal and animal product movements. Because the scrapie agent elicits no detectable antibody response in the host, vaccines and serological tests have not been possible, although great progress is being made in the area of diagnostics. Current diagnostic tests being used for sheep and goats are not validated for the

preclinical live animal (Figure 4.2). This ineffectiveness has prohibited ascertaining which animals are incubating the disease and may be shedding the agent until the onset of clinical signs. This inability to identify apparently normal sheep that may be shedding the agent makes prohibiting all movement of sheep and goats and their products into an area the only absolute way to prevent introduction of scrapie.

If research finds that certain genotypes are resistant to scrapie infection or reduce the transmission of scrapie, genetic testing would prove very useful to a flock owner. There is evidence that breeding for certain PrP genotypes will decrease, if not eliminate, clinical disease. Research, however, has not been completed to eliminate the possibility of a "carrier" animal.

Until some of the new preclinical tests are validated, the ideal means for preventing the introduction of scrapie is to maintain a closed flock, especially with regard to ewes. Any replacement ewes or breeding rams should originate from flocks known not to be affected with scrapie and have management practices precluding its introduction. However, in reality, this procedure may be difficult to accomplish, because there is no definitive prepurchase test to assure an animal is disease free. Thus, a buyer must rely on the seller's knowledge, integrity, and honesty.

Clinical disease reporting can be influenced by many factors. One of the most important is the knowledge level of producers and practicing veterinarians. Producers and veterinarians must be aware that scrapie is a reportable disease and that veterinarians know where to report it. They also must be familiar with its clinical signs and how to differentiate scrapie from other diseases. Hence, education is an essential component for scrapie surveillance.

Chronic Wasting Disease

Elizabeth S. Williams

Clinical Signs

Chronic wasting disease naturally affects mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*) (Spraker et al. 1997; Williams and Young 1992). Affected cervids, i.e., members of the deer family, are older than 17 months of age; most are 3 to 5 years old, but may be older. Gender does not seem to affect susceptibility to CWD. The earliest clinical signs are behavioral changes that may include alterations in interaction with humans and members of the
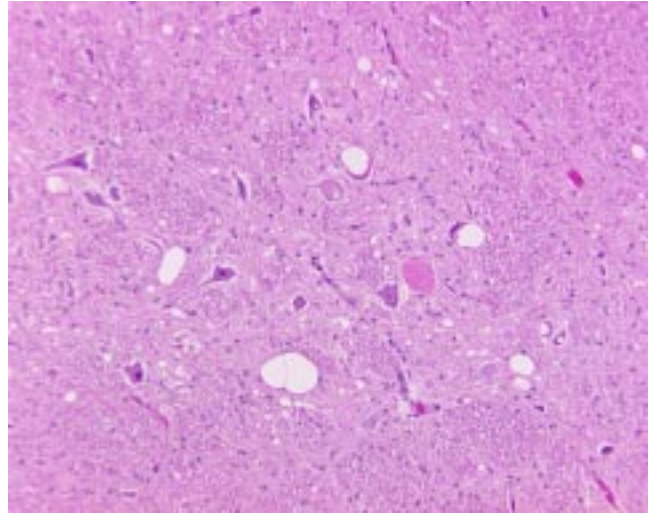
Figure 4.2. Nucleus of the spinal tract of the trigeminal nerve in the medulla oblongata of a sheep with scrapie. Vacuoles are present within neuron cell bodies and in the neuropil. Routine hematoxylin and eosin stain. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

herd. These subtle changes are often only appreciated by caretakers familiar with individual animals. With disease progression, behavioral alterations may include periods of stupor and depression. As the name suggests, progressive weight loss is characteristic of CWD and may occur over a long period (Figure 4.3). Duration of clinical signs varies from a few days in unusual cases to as long as a year but is most often 2 to 3 months. At the terminal stages of disease, animals are emaciated. However, intercurrent disease,

Figure 4.3. Mule deer (*Odocoileus hemionus*) showing clinical signs of chronic wasting disease including emaciation. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

Figure 4.4. Mule deer (*Odocoileus hemionus*) showing clinical signs of chronic wasting disease including excessive salivation. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

Epidemiology

CWD was recognized as a syndrome by biologists working with captive deer in the late 1960s. It occurs in deer and elk on a few wildlife research facilities and in free-ranging cervids in limited areas of ten contiguous counties in southeastern Wyoming and north central Colorado. Recently, CWD was diagnosed among privately owned elk on game farms in Saskatchewan, South Dakota, Nebraska, Montana, Colorado, and Oklahoma. Fewer than 250 cases of CWD have been diagnosed over the last 20 years, mostly in captive cervids from research facilities and in free-ranging mule deer.

Surveillance of free-ranging deer and elk for evidence of CWD has taken two forms. Wild cervids showing clinical signs compatible with the case definition are examined in veterinary diagnostic laboratories for spongiform encephalopathy. No cases of clinical CWD in free-ranging cervids have been diagnosed outside the known endemic areas of Wyoming and Colorado. The second surveillance technique involves voluntary or mandatory submission of heads of hunter-harvested deer and elk so that brains can be examined for evidence of preclinical/subclinical CWD. Hunter-harvested cervid surveillance began in 1983, and thousands of animals have been tested. In core management units in the endemic area, estimated prevalence is 6 to 8% in mule deer and less than

any other disease that modifies the current disease, especially aspiration pneumonia, may cause an affected animal to die while still in good to fair body condition. In the later stages of CWD, clinical signs may include increased drinking and urinating, excessive salivation (Figure 4.4), and incoordination and trembling. These clinical signs are nonspecific and could be caused by many other diseases affecting wild and captive deer and elk. Thus, laboratory examination is required to diagnose CWD (Figures 4.5–4.6).
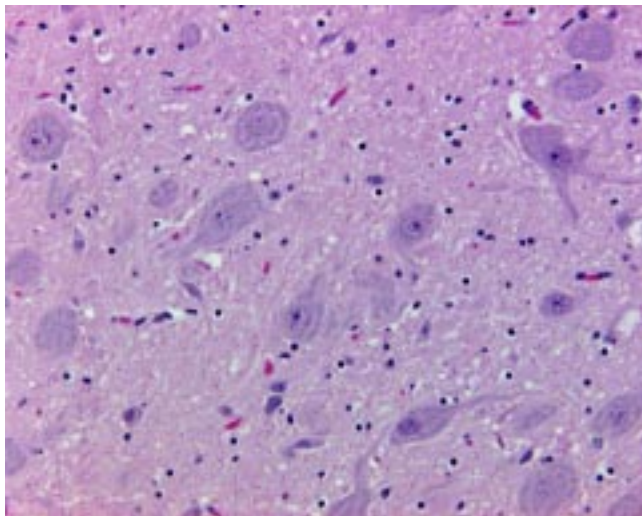

Figure 4.5. Photomicrograph of the dorsal motor of a normal mule deer (*Odocoileus hemionus*). Note there are no vacuoles within the neuropil or neurons within this section. Routine hematoxylin and eosin stain. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.
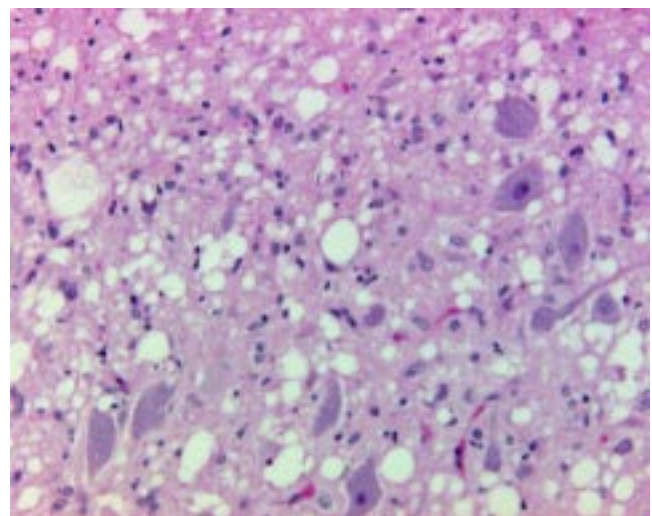

Figure 4.6. Photomicrograph of the dorsal motor nucleus of a mule deer (*Odocoileus hemionus*) with chronic wasting disease. Note the vacuoles within the neuropil and neurons in this nucleus of the brain. Routine hematoxylin and eosin stain. Photograph courtesy of Dr. Terry Spraker, College of Veterinary Medicine, Colorado State University, Fort Collins.

1% in elk. In surrounding management units, estimated prevalence in both species is under 1%. The epidemiology and prevalence of CWD in privately owned elk is under study.

CWD's mode of transmission is unknown. Epidemiologic evidence strongly suggests that lateral transmission occurs among deer and elk (Miller, Wild, and Williams 1998) and probably from mule deer to elk and white-tailed deer. Although maternal transmission may occur, it does not explain many cases of CWD. Concentration of animals in captivity may facilitate transmission; however, CWD is maintained in populations of deer and elk even at moderate to low population densities. There is no evidence that CWD is a foodborne disease associated with consumption of animal protein. The origin of CWD is not known, and the source(s) of CWD in captivity and in the wild is uncertain. Based on strain typing in mice, CWD is not the same as BSE or known scrapie strains (Bruce et al. 1997).

The known host range of CWD is mule and white-tailed deer and Rocky Mountain elk. Subspecies of *Cervus elaphus* are probably susceptible, but it is not known if other cervids can develop CWD. There is currently no evidence that other wild species, domestic animals, or humans are susceptible to CWD, though research to better characterize the host range is currently underway.

Prevention and Control

To prevent the geographic spread of CWD, free-ranging deer and elk are not transplanted or moved from the endemic areas of Wyoming and Colorado. Surveillance to monitor distribution and prevalence in free-ranging deer and elk is being conducted so that changes over time can be detected. Because disease transmission may be facilitated by high densities of deer and elk, concentration of these species by artificial feeding is either not done or prohibited in CWD endemic areas.

Guidelines are being developed by the elk-farming industry, in conjunction with state and federal animal health and wildlife management agencies, to address CWD surveillance, quarantine, inventory, and diagnosis in privately owned elk. Under state animal or provincial health agency authority, CWD-affected privately owned elk herds have been quarantined or depopulated.

Education of the public, hunters, meat processors, taxidermists, wildlife biologists, game wardens, animal health officials, and veterinary diagnosticians and pathologists is being conducted through brochures, press releases, journal articles, public presentations, workshops, and a video. Because of the many unknowns surrounding CWD, much research is underway to better characterize the disease, determine its host range, develop and validate diagnostic tests, and understand its epidemiology. Results will be used to develop methods for prevention and control.

Transmissible Mink Encephalopathy
Jason C. Bartz and Doris Olander

Distribution and Clinical Signs

Transmissible mink encephalopathy (TME) is a TSE of ranch-raised mink that was first identified in Wisconsin in 1947. TME has occurred in the United States in 1961, 1963, 1964 and most recently in 1985 in Stetsonville, Wisconsin (Hadlow and Karstat 1968; Hartsough and Burger 1965; Marsh et al. 1991). Outside of the United States, TME has been observed in ranch-raised mink in Ontario, Canada, Finland, the former East Germany, and the former Soviet Union (Danilov, Bukina, and Akulova 1974; Hadlow and Karstat 1968; Hartung, Zimmerman, and Johannesen 1970). Early clinical signs include subtle alterations in normal behavior patterns, cleanliness, and difficulty in eating. As the course of disease progresses, affected mink demonstrate incoordination (ataxia) and abnormal tail carriage over the back.

Etiology

Outbreaks of TME have been associated with contaminated feedstuffs. For example, the 1961 outbreak of TME occurred on five mink ranches in Wisconsin that shared a common commercial source of a ready-mix feed ration. In 1963, an outbreak of TME in two mink ranches in Wisconsin was linked to the use of beef carcasses that were unfit for human consumption ("downer cows") in the mink rations. In 1965, a retrospective study established that TME is a foodborne disease with an incubation period ranging from 7 to12 months (Hartsough and Burger 1965).

The source of the TSE agent responsible for TME, however, remains elusive. Originally, it was assumed that sheep scrapie was the source of TME. Several lines of experimental and epidemiological evidence are inconsistent with this idea. Although mink will succumb to scrapie after intracranial inoculation, at-

tempts to orally transmit U.S. and U.K. strains of sheep scrapie to mink have not been successful (Marsh and Hanson 1979). This has been interpreted as evidence that scrapie is not the source of TME. It cannot be ruled out, however, that a rare strain of scrapie that was not included in this study is pathogenic for mink. Epidemiological data also has raised doubt about the hypothesis that sheep scrapie is the source of TME. The 1963 outbreak of TME in Wisconsin was linked to a common source of bovine tissue in the feed (Hartsough and Burger 1965). In the 1963 TME outbreak in Ontario, the mink rancher stated that sheep tissue had not been fed to the mink (Hadlow and Karstat 1968). More recently, the outbreak of TME that occurred in Stetsonville, Wisconsin in 1985 provides further circumstantial evidence about the origins of TME (Marsh and Harsough 1985; 1988). The mink rancher primarily fed "downer"cows to the mink with no record of sheep contact, which implicated cattle as the source of the TSE agent (Marsh et al. 1991). Mink have been fed BSE-infect-ed tissue and subsequently developed neurological disease 15 months after oral feeding (Robinson et al. 1994). This is the first demonstration of oral infection of mink with a TSE from a naturally infected ruminant species and suggests that mink are more susceptible to BSE than sheep scrapie by the oral route.

Current experimental and epidemiological data suggest that a cattle TSE, and not sheep scrapie, may be the source of the TSE agent responsible for TME. How can this be reconciled with the fact that a cattle TSE has not been observed in the United States? The limited number of recorded outbreaks of TME suggests that if a cattle TSE is responsible for TME, it is rare. It has been determined that the occurrence of cattle TSEs in the United States at the rate of 1 in 975,000 adult cattle per year could be sufficient to produce the number of observed TME outbreaks (Robinson 1996). At this low rate, if a cattle TSE exists at all, it would be at the limits of detection of current surveillance programs.

5 Transmissible Spongiform Encephalopathies in Humans

Ermias D. Belay, Morris E. Potter, Lawrence B. Schonberger

Creutzfeldt-Jacob Disease

The most common form of TSE in humans is CJD. It occurs with an estimated annual incidence of one case per million population in many countries, including the United States (Holman et al. 1996). Affected patients usually present with memory impairment; mental deterioration; abnormalities in walking, balance, or speech; or visual disturbances. As the disease progresses, patients often develop tremors and involuntary jerking movements and eventually become bed-ridden. About two-thirds of CJD patients develop the disease between the ages of 55 and 75 years (Brown et al. 1986). The median age of CJD decedents in the United States is 68 years (Holman et al. 1996). CJD is a rapidly progressive and invariably fatal disease; more than half the patients die within 6 months after the appearance of clinical signs, and 85 to 90% of patients die within 12 months of illness onset (Brown et al. 1986). In 75 to 85% of CJD patients, serial electroencephalographic (EEG) recordings show a characteristic pattern that, in conjunction with the typical clinical features, is considered to be diagnostic with over 95% accuracy (Will 1996). A new diagnostic test that detects the presence in the cerebrospinal fluid of a protein that seems to be a marker for CJD has been developed. In patients with dementia, the sensitivity and specificity of this test in detecting CJD has been reported by a research laboratory to be 96% (Hsich et al. 1996). Pathologic examination of brain tissue obtained at biopsy or autopsy provides the most definitive, common diagnostic test for CJD.

CJD occurs as a sporadic, familial, or iatrogenic (medically induced) disease. Sporadic CJD accounts for about 85% of CJD cases; the disease is considered sporadic because no recognizable pattern of disease transmission has been reported in these patients. Familial CJD, an inherited form of the disease, accounts for 5 to 15% of CJD patients and has been associated with specific mutations of the PrP gene. Although familial CJD can occur in a seemingly sporadic pattern, often, there is a family history of CJD. In a few U.S. cases, iatrogenic transmission of the CJD agent has been reported. Most of these cases have occurred among persons who received pituitary-derived growth hormone. Several iatrogenic cases have been associated with the use of cadaveric dura mater and corneal grafts (Brown 1996).

Gerstmann-Sträussler-Scheinker Syndrome

Gerstmann-Sträussler-Scheinker syndrome is a heterogeneous, familial form of CJD usually characterized by balance and gait abnormalities, infrequent jerking movements, an atypical EEG, a prolonged duration of illness (average, 3.4 to 8.8 years), and a brain pathologic feature of numerous amyloid plaques. GSS is uniformly fatal. GSS occurs in association with various specific genetic mutations at different sites of the prion protein gene, which may correlate with various clinical characteristics (Ghetti et al. 1996). GSS occurs at a rate that is 10 to 20 times lower than that of sporadic CJD. Familial clusters of GSS have been reported in North America, Europe, Israel, and Japan.

Fatal Familial Insomnia

Fatal familial insomnia, a TSE that primarily occurs as an inherited disease, shares a similar prion protein gene mutation with familial CJD. However, patients with FFI have a distinct clinical manifestation, brain pathologic profile, and specific polymorphism at codon 129 of the gene that codes for PrP. Affected patients predominantly present with severe sleep disturbances in association with speech abnormalities, tremor, or involuntary jerking movements. Patients with FFI also may exhibit some disturbances of the nervous system that regulate body temperature, heart rate, and blood pressure. The brain pathologic lesion in FFI patients is largely confined to a specific part of the brain known as the thalamus (Manetto et al. 1992). FFI has been reported in families from Australia, Austria, Britain, France, Germany, Italy, Japan, and the United States.

New Variant Creutzfeldt-Jakob Disease

New variant Creutzfeldt-Jakob Disease was first proposed as a distinct clinicopathologic entity in April 1996 when Will and colleagues (1996) reported 10 unusually young patients with CJD who had atypical clinical features and an apparently unique brain pathologic profile. The age at the time of onset of these patients ranged from 16 to 39 years (median, 28 years) (Schonberger 1998; Will et al. 1996). The typical clinical picture for nvCJD in the 10 patients included prominent behavioral changes at the time of clinical presentation, with subsequent onset of neurologic abnormalities, including muscle incoordination within weeks or months, dementia, and involuntary jerking movements late in the illness, a duration of illness of at least 6 months, and nondiagnostic EEG changes. The unique brain pathologic profile - in addition to the presence of spongiform encephalopathy - included the presence of numerous daisy-shaped pathologic lesions in several parts of the brain consisting of amyloid plaques surrounded by spongiform changes. Review of medical and family histories and genetic analyses of the patients did not provide an adequate explanation for the occurrence of the cluster (Schonberger 1998; Will et al. 1996). The subsequent occurrence of additional nvCJD cases in the United Kingdom and accumulating laboratory evidence have strongly supported a conclusion that nvCJD resulted from the transmission to humans of the agent causing BSE (Belay 1999; Schonberger 1998). As of September 4, 2000, 82 definite and probable cases of nvCJD have been officially reported in the United Kingdom, three in France, and one in the Republic of Ireland. Despite increased surveillance efforts, no nvCJD case has been reported in the United States or in other countries where no BSE has been identified (Schonberger 1998).

6 Relationship Between Transmissible Spongiform Encephalopathies in Animals and Humans

Ermias D. Belay, Morris E. Potter, Lawrence B. Schonberger

Dietary Risk Factors in Creutzfeldt-Jakob Disease

Since CJD was first reported to be experimentally transmissible by intracerebral inoculation in chimpanzees (Gibbs et al. 1968), speculations have persisted about the possibility that natural sources of infection could account for the sporadic form of CJD. Evidence supporting specific natural sources of infection, however, remains weak. For example, a report of an association between CJD and eating squirrel brains was based on histories from just five CJD cases in Kentucky, a state where the incidence of CJD is not increased. This report also acknowledged the absence of a documented spongiform encephalopathy in squirrels (Berger, Weisman, and Weisman 1997). In the 1970s, the higher incidence of CJD in Sephardic Jews had raised concerns that CJD in Jewish families of North African origin may have been associated with scrapie because of their custom of eating sheep brain. However, subsequent studies demonstrated that the Sephardic families were affected with an inherited form of familial CJD that was associated with a PrP gene mutation (Gajdusek 1996). The relatively stable incidence of sporadic CJD in many countries, despite variations in the incidence or absence of scrapie, does not support a link between scrapie and sporadic CJD (Will et al. 1999). The absence of CJD in persons 5 to 19 years of age in the United States during the 1979–1997 mortality surveillance period, despite widespread exposure of children to a variety of animal products, provides some reassurance about the safety of such products (Schonberger et al. 1999). This is in contrast to nvCJD, which is believed to have resulted from consumption of BSE-infected cattle products. In the United Kingdom, seven (13%) of the first 52 patients with nvCJD died before their 20th birthday (Will, pers. comm. 2000).

Several case-control studies that examined consumption of organ meat, including animal brain, liver, and kidney, as possible sources of infection in sporadic CJD patients have not indicated a consistent association (Belay 1999). A recent re-analysis of pooled data from three previous case-control studies did not show a significant association of CJD with consumption of organ meat, including brain, liver, and kidney (Wientjens et al. 1996). A large case-control study conducted in several European countries showed no significant association overall of CJD with consumption of beef, veal, lamb, or pork or with occupational exposure to animals or animal products, including butchers and slaughterhouse and farm workers (Van Duijn et al. 1998). In this study, consumption of raw meat and brain were significantly associated with an increased risk of CJD. After a conditional regression analysis, however, the association of CJD and brain consumption was no longer significant. In the European study, epidemiologic evidence for a possible dietary risk factor for CJD seemed strongest for consumption of raw meat, although this association has not been observed in other studies and would need to be confirmed. Published case-control studies have enrolled fewer than 750 CJD cases, and have many inherent limitations, including the need to use family members or other surrogates for case information about potential exposures that occurred many years earlier. On balance, the available data do not provide compelling evidence for a relationship between animal TSEs and classic CJD in humans.

New Variant Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy

In contrast to the situation related to classic CJD, there is strong epidemiologic and laboratory evidence for a causal association between nvCJD and BSE. Although the precise mode of transmission of the BSE agent has not been described, dietary exposure through consumption of contaminated cattle products seems most likely. The absence of nvCJD in geographic locations free of BSE supports a causal association. In addition, the interval between the most likely period for an initial extended exposure of the population to potentially BSE-contaminated food (1984–1986) and onset of initial nvCJD cases (1994–1996) is consistent with known incubation periods for

CJD (Schonberger 1998). An experimental study reported in June 1996 showed that three cynomologous macaque monkeys, the closest animal model to humans, inoculated with brain homogenates obtained from cattle with BSE had clinical and brain pathologic features strikingly similar to nvCJD (Lasmezas et al. 1996). A subsequent U.K. laboratory study indicated that the molecular characteristics of infecting prions obtained from 10 nvCJD patients were similar to prions obtained from BSE-infected animals, including cattle, mice, domestic cat, and macaque; these molecular characteristics were distinct from those of prions obtained from patients with classic CJD (Collinge et al. 1996).

Most recently, interim results of an ongoing exper-imental study involving inoculation of a panel of inbred mice with the agents causing BSE and nvCJD substantially increased the strength of the scientific evidence for a causal association between nvCJD and BSE (Bruce et al. 1997). Two additional groups of inbred mice and a group of cross-bred mice inoculated with brain homogenates from nvCJD cases were also reported to have had latency periods and lesion profile consistent with the BSE pattern (Bruce, pers. comm. 2000). The latency period, neuropathology, and disease-causing PrP isoforms in transgenic mice expressing bovine PrP that were inoculated with nvCJD, BSE, and scrapie brain extracts provided additional evidence supporting the link between BSE and nvCJD (Scott et al. 1999).

7 Transmissible Spongiform Encephalopathies in the United States

Linda A. Detwiler

Bovine Spongiform Encephalopathy

No BSE case has ever been detected in the United States. In an attempt to maintain this status, the USDA's APHIS has taken a series of preventive measures and implemented an aggressive surveillance system (U.S. Department of Agriculture 1999a). These measures include prohibitions and/or restrictions on certain animal and product imports, ongoing surveillance for signs of the disease, preparation of an emergency response plan in the event of an introduction, and educational efforts. The APHIS actively shares information and coordinates closely with other federal agencies — as well as with states, livestock and affiliated industries, veterinary and research communities, and consumer groups — to ensure that the United States has a uniform approach to TSEs that is based on sound scientific information.

In 1989, the United States prohibited the importation of ruminants and most ruminant products from countries affected with BSE. APHIS has conducted a trace-back effort to locate each of the 496 cattle from the United Kingdom or Ireland that were imported into this country between January 1, 1981 and July, 1989. Only 4 of these animals are known to be alive; they are under quarantine and monitored by APHIS personnel. Two cows imported from Belgium in 1996 and 33 from other European countries are also under quarantine. No evidence of BSE has been found in any of these imported animals.

On December 12, 1997, APHIS prohibited importation of live ruminants and most ruminant products from Europe, pending a thorough risk assessment. There was evidence that European countries have had high risk factors for several years and less-than-adequate surveillance.

In May 1990, the United States began an aggressive and active BSE surveillance program to ensure timely detection and swift response in the unlikely event that an introduction were to occur. This surveillance program incorporates both locating imports from the United Kingdom or other countries with detected BSE and targeting active and passive surveillance for either BSE or another TSE in cattle.

More than 250 federal and state regulatory veterinarians are specially trained to diagnose foreign animal diseases, including BSE. Several agencies are involved in the surveillance program, including the FSIS, with APHIS as the lead agency. The USDA, in cooperation with the livestock and allied industry groups, has been educating producers; private, university, and regulatory veterinarians; slaughterhouse and market owners; and others associated with the livestock industry about BSE since the late 1980s. Education is an essential component for a sound surveillance system.

Samples of brain for BSE surveillance of adult cattle are obtained from (1) field cases of cattle exhibiting signs of neurological disease, (2) cattle condemned at slaughter for neurological reasons, (3) rabies-suspect cattle submitted to public health laboratories, (4) neurological cases submitted to veterinary diagnostic laboratories and teaching hospitals, and (5) random sampling of aged cattle (predominantly dairy) that are nonambulatory at slaughter.

As of August 31, 2000, a total of 11,374 brains had been examined for BSE or another form of TSE in cattle in the United States. No evidence of either condition has been found (U.S. Department of Agriculture 2000b).

Assessing the Effectiveness of Current Surveillance

Analyses were performed in 1991 to assess the risk factors associated with BSE. These risk factor analyses continue to demonstrate that the overall risk of BSE in the United States, based on what is known about TSEs, is extremely low and is decreasing. These risk analyses also have been used to identify the portion of the cattle industry that would be at the highest risk of contracting BSE. This population is where the majority of the active surveillance (brain submission and examination) has occurred. Specifically, this surveillance has been targeted at adult animals that demonstrated neurological abnormalities or animals that were nonambulatory at slaughter. No evidence

of BSE has been found in this sampling from the highest risk population, either through histological examination or immunohistochemistry.

Statistical calculations can be used to define the maximal incidence of BSE in the United States at current surveillance levels. These calculations are based on the following assumptions:

1. The U.S. dairy cow inventory is 9.4 million, beef cow inventory is 35.6 million, and the total number of adult cows and bulls is 47.4 million.
2. An estimate of cows exhibiting central nervous system (CNS) abnormalities can be extrapolated from the National Animal Health Monitoring System (NAHMS) Dairy 96 Study. This study asked producers to answer a question about the number of cows that died on the farm as a result of lack of coordination or severe depression. This estimate is 0.1%.
3. In the United States, 750 to 1,000 cattle are sampled annually.

The fact that no evidence of BSE has been found in the 11,374 bovine brains so far examined provides the basis for an estimate of the maximal potential incidence of BSE in the United States. Assuming that one samples from the population of adult dairy cows with CNS signs and that the disease is expected to occur only in this population, the size of the population from which samples are taken would be 9,400 (0.1% of 9.4 million). Given that 750 animals are sampled annually and no BSE has been detected, the maximal number of diseased animals in the U.S. population would be 37. If this number is divided by the total population of all adult cows and bulls, the maximal incidence would be 0.7 per million. This figure is at 95% confidence. If the sample is drawn from the population of adult beef and dairy cows with CNS signs, the maximal incidence would be 3 per million. The risk of disease in the beef cow population would be expected to be lower than that in the dairy cow population, because of differences in management practices. Therefore, this figure can be assumed to be slightly high.

General Surveillance

In addition to this active surveillance, general surveillance takes advantage of existing data sources. These sources include a database maintained at Purdue University with diagnoses submitted from 27 U.S. veterinary schools, CNS antemortem condemnation data from FSIS, necropsies performed at zoos on various species, and a veterinary diagnostic laboratory reporting system. Referrals of unusual cases by private practitioners to veterinary schools and diagnostic laboratories provide additional surveillance. Through these sources, there has been no reported appearance of a new neurologic disease in cattle nor does there seem to be an increase in the number of neurologic diagnoses or referrals. Furthermore, no cases of any of the other BSE-related TSEs (FSE, TSE of exotic ruminants and cats) have been diagnosed in the United States.

Laboratory Procedures

Brain submissions to the USDA's National Veterinary Services Laboratory (NVSL) in Ames, Iowa are evaluated by using both histological examination and IHC detection for PrP^{res}. The NVSL began using IHC in 1993 and since then has increased the percentage of samples tested with this method. Initially, IHC was used in cases where an endemic disease could not be ruled out. The protocol is to evaluate all samples submitted to NVSL by histology and IHC. In addition, state diagnostic laboratories are asked to forward samples for IHC or western blot analysis from neurological cases where domestic diseases cannot be ruled out.

Incentives

The USDA and state departments of agriculture in cooperation with members of the National Renderers' Association pay for the transportation and disposal of carcasses from suspect cattle with neurological disease submitted for BSE surveillance. This payment is an incentive for producers and market owners, as they would otherwise have had to bear the costs of carcass disposal.

Feeding and Management Practices

The initial risk analyses done by APHIS in 1991 described significant differences in feeding and management practices between the United States and the United Kingdom that are believed to be important in decreasing the risk of BSE (U.S. Department of Agriculture 1992a). The United States feeds more concentrates per animal and has an abundance of available plant-based proteins such as soybean meal and cottonseed meal. Therefore, the portion of animal proteins used as a percentage of all major feed proteins has been significantly less in the United States. Another critical difference was the inclusion of meat and

bone meal as a protein source in calf starter feeds in the United Kingdom prior to the feeding bans. Comparable U.S. feeds for young calves contain plant-based proteins.

The initial U.S. risk analyses further examined the sheep scrapie origin hypothesis (U.S. Department of Agriculture 1992b). The United States has an abundance of cattle and relatively few sheep — approximately 7 million sheep to 100 million cattle. This ratio of sheep to cattle is in contrast to the situation in the United Kingdom, where the ratio of sheep to cattle is 32 times greater. Sheep make up approximately 1.5% of ruminant meat production in the United States, with mature sheep accounting for about 6% of sheep slaughter. The amount of sheep offal is approximately 0.6% of all U.S.-rendered product, compared with an estimate of 14% of U.K.-rendered product. The amount of mature sheep offal is approximately 0.1% of all U.S.-rendered product. To measure potential risk from sheep meat and bone meal, the ratio of dairy concentrate fed per mature sheep meat and bone meal produced must be considered. A larger ratio means a greater dilution and therefore less potential risk. In the United States, the ratio is 34,760:1 or over 17 tons of dairy concentrate fed for each pound of sheep meat and bone meal produced. This ratio is in contrast to the figures from the United Kingdom, where the ratio was 778:1. The results also indicated geographical variation in the ratio of sheep to cattle raw materials for rendering; however, very few areas in the United States approached rations similar to those in the United Kingdom.

To further reduce the risks associated with animal feeds, a U.S. FDA regulation that prohibits feeding most mammalian protein to ruminants went into effect August 4, 1997. It is Title 21, CFR, Part 589.2000. These are very prudent measures, given the remote possibility that BSE exists in the United States.

Scrapie

The first case of scrapie was diagnosed in the United States in 1947. This disease was in a sheep of British origin imported through Canada. From then until August 1, 1999, scrapie has been confirmed in 950 flocks. The majority of scrapie (87%) has been confirmed in Suffolk sheep. Other breeds in the United States that have been diagnosed with scrapie are Hampshire, Border Leicester, Cheviot, North Country Cheviot, Corriedale, Cotswold, Dorset, Finn, Merino, Montadale, Rambouillet, Southdown, Shropshire, and various crossbreeds. There have been seven cases of natural scrapie diagnosed in goats in the United States. The first five cases diagnosed in goats were all sheep-associated and did not occur in herds limited to goats.

Surveillance in the United States depends heavily on educational efforts. Education of producers and veterinarians enhances reporting of scrapie. Tracebacks to source and exposed flocks are an integral part of the U.S. surveillance program.

Scrapie diagnosis is primarily based on the occurrence of clinical signs of the disease and must be confirmed by laboratory testing. Histopathological examination of brain tissue collected after the animal dies or is euthanized may be conducted on tissues sent directly to the USDA's NVSL or may first be conducted by other diagnostic laboratories. Samples of brain tissue also routinely are tested at NVSL by using IHC. IHC has helped to confirm up to one-third of the submitted cases, which could not have been diagnosed by histology alone. Western blotting is used in specific circumstances. The combination of techniques has allowed diagnosis in cases that are histopathologically inconclusive or where the brain has been autolyzed or frozen. Research is underway to develop a test that can be used to detect the scrapie agent or a marker of infection in the living animal.

The USDA's APHIS and Agricultural Research Service (ARS) are conducting studies to evaluate and validate the testing of peripheral tissues to detect scrapie in the preclinical or subclinical small ruminant. The evaluation will examine various tissues — including tonsil, lymph nodes, nictitating membrane, and brain — from lambs less than 1 year of age and from clinically normal mature sheep at slaughter. Tissues are being tested by IHC and western blotting. If this proves effective, such testing may be useful as a screening tool and to facilitate tracebacks to infected flocks and may also allow prevalence to be evaluated.

The type of scrapie control program has influenced the amount of scrapie that has been detected, because of changes in the severity of the program and amounts of indemnity paid. Efforts to eradicate scrapie in the United States have been in effect since 1952, when the Secretary of Agriculture declared a state of emergency to deal with the disease. At that time, once the disease was confirmed, the flock was quarantined and then depopulated. All exposed sheep sold from the flock were traced and slaughtered. In 1957, the regulations were amended to include location and subsequent depopulation of source flocks and animals sold from them. Although this approach was modified over

time, the primary focus remained total flock depopulation.

In 1983, emphasis shifted away from total flock depopulation. The bloodline surveillance program adopted at that time required that the maternal bloodlines of a scrapie-infected sheep or goat be removed from the flock/herd. A 1985 review of the bloodline surveillance program had two conclusions: (1) bloodline surveillance should be abolished because it was not effective; lateral transmission within flocks was significant and scientific knowledge was inadequate to effectively eradicate the disease, and (2) the USDA should redirect its efforts and funding toward education and research.

The negotiated rulemaking process was used to develop a replacement for the bloodline surveillance program. This process brought together sheep producers, allied industry representatives, state veterinarians, scientific advisors, and APHIS officials to negotiate the text of a new program. The rulemaking process resulted in the initiation of a Scrapie Flock Certification Program and regulations to restrict the interstate movement of high-risk sheep from known infected and source flocks.

The intent of the Scrapie Flock Certification Program is to monitor flocks over a period of five years or more and to identify those that are free of scrapie. Because there is no live-animal test and scrapie has a long incubation period, a flock is considered free of the disease if no sheep have been diagnosed with scrapie and there is no evidence over an extended period of time. The program provides participating owners with the opportunity not only to protect their sheep from scrapie but also to enhance the marketability of their animals. The control effort focuses on risk reduction and sound husbandry practices. Because each advancing phase represents a lower risk of scrapie in the flock, the economic value of the animals is increased, especially after completing the 5-year program and attaining "certified" status. This program also may have implications for exporting breeding stock.

If infection is found in a flock, an epidemiological investigation will be conducted. This investigation will trace and identify source flocks and exposed animals. A flock plan will be developed and implemented to rid an infected or source flock of scrapie. They are developed by the flock owner or manager, regulatory personnel, and an accredited veterinarian. Flock plans include (1) thorough epidemiological investigation, (2) identification and removal of high-risk animals, (3) cleaning and dis-

infection of the premises, (4) identification of the breeding flock, and (5) record keeping.

In 1997, the sheep industry felt there was a need for further regulations to strengthen current scrapie control measures. They asked APHIS to publish an ANPR to solicit comments on the best approach for scrapie control. The ANPR was published in January 1998, and eventually led to an update of the Certification Program effective September 1999 (U.S. Department of Agriculture 1999b). Rules on interstate movement of sheep and goats as well as on pilot projects to evaluate flock cleanup plans based on testing have been proposed (U.S. Department of Agriculture 2000a). The proposed rule was published in November 1999.

Other Animal Transmissible Spongiform Encephalopathies

Chronic Wasting Disease

CWD was first recognized in captive mule deer (*Odocoileus hemionus*) housed at a Colorado research facility in 1967. A similar condition was diagnosed in captive deer at a Wyoming research facility in 1978. The disease also was confirmed in Rocky Mountain elk (*Cervus elaphus nelsoni*). In addition to the cases at the research facilities, CWD has primarily been confined to free-ranging deer and elk in a 10-county endemic area in north central Colorado and southeastern Wyoming. It has recently been detected in five privately-owned elk herds in South Dakota, Nebraska, Colorado, Montana, Oklahoma, and Saskatchewan.

Although CWD shares many clinical and pathological characteristics with the other TSEs such as scrapie and BSE, strain typing of tissues from a mule deer with CWD indicates that it may be a separate disease entity and not caused by the same strain of agent as BSE or scrapie (Bruce et al. 1997).

Surveillance for CWD in free-ranging deer and elk in Colorado and Wyoming has been ongoing since 1983, respectively, and has confirmed that the disease is confined to endemic areas. An extensive nationwide surveillance effort was started during 1997–1998 to better define the geographic distribution of CWD. This surveillance effort is a two-pronged approach consisting of hunter harvest cervid surveys conducted in many states, as well as surveillance throughout the entire country targeting deer and elk exhibiting clinical signs sug-

gestive of wasting disease. In the free-ranging population, there have been approximately 95 positive animals identified, all of which have been from the endemic area. In addition, over 5,000 samples from outside the endemic area have demonstrated no evidence of CWD. Again, no free-ranging that did not originate from the endemic areas animals have been found to be positive.

Transmissible Mink Encephalopathy

TME was first diagnosed in the United States in 1947. To date, five U.S. outbreaks have been recorded, in 1947, 1961, 1963 (two outbreaks), and 1985. Research and surveillance continue at the University of Wisconsin.

Appendix A: Symbols, Acronyms, and Abbreviations

ANPR	Advanced Notice of Public Rulemaking	IHC	immunohistochemistry
APHIS	Animal and Plant Health Inspection Service	kDa	kilodalton
ARS	Agricultural Research Service	NAHMS	National Animal Health Monitoring System (APHIS)
BSE	bovine spongiform encephalopathy		
CDC	Centers for Disease Control and Prevention	nm	nanometer
CFR	Code of Federal Regulations	NVSL	National Veterinary Services Laboratory
CJD	Creutzfeldt-Jakob disease	PrP	prion protein
nvCJD	new variant Creutzfeldt-Jakob disease	PrP^c	normal prion protein
CNS	central nervous system	PrP^{res}	transmissible spongiform encephalopathy-specific form of prion protein (equivalent to PrP^{Sc})
CWD	chronic wasting disease		
DNA	deoxyribonucleic acid	PrP^{Sc}	scrapie form of prion protein (equivalent to PrP^{res})
EEG	electroencephalograph	PrP^{sen}	same as PrP^c
FDA	U.S. Food and Drug Administration	RNA	ribonucleic acid
FFI	fatal familial insomnia	SAF	scrapie-associated fibrils
FSE	feline spongiform encephalopathy	Sip	scrapie incubation period
FSIS	Food Safety Inspection Service	TME	transmissible mink encephalopathy
g	gram	TSE	transmissible spongiform encephalopathy
GSS	Gerstmann-Sträussler-Scheinker syndrome	USDA	U.S. Department of Agriculture

Appendix B: Internet Sites for TSE Occurrence Data

Latest numbers and general information on transmissible spongiform encephalopathies can be found at the Internet sites listed below (current as of print date).

U.S. surveillance numbers:
<http://www.aphis.usda.gov/oa/bse/bsesurvey.html>

U.K. Ministry of Agriculture, Fisheries and Food BSE site and surveillance numbers:
<http://www.maff.gov.uk/animalh/bse/index.html>

World surveillance numbers other than the United Kingdom (The U.K. site is more current for the U.K. numbers.):
<http://www.oie.int/Status/A_bse.htm#autres>

U.K. Department of Health for CJD/nvCJD numbers in the United Kingdom only:
<http://www.doh.gov.uk/cjd/cjd_stat.htm>

U.S. Department of Agriculture BSE information:
<http://www.aphis.usda.gov/oa/bse/>

U.K. Department of Health TSE page:
<http://www.doh.gov.uk/cjd/cjd1.htm>

New variant Creutzfeld Jakob Disease case numbers outside of the United Kingdom: none available

U.S. CJD surveillance:
<http://www.cjdsurveillance.com>
<http://www.cdc.gov/ncidod/diseases/cjd/cjd.htm>

Appendix C: Nature of Infectious Agent

Richard Rubenstein

Virus Hypothesis

One of the arguments surrounding the debate on whether or not the agent is a virus is its unusual resistance to treatments, i.e., radiation, formalin, heat, sodium hypochlorite, that inactivate conventional viruses. However, this resistance does not imply total insensitivity to these treatments. In fact, studies have indicated a 90 to 99% loss of infectivity following these treatments (Alper et al. 1967; Brown et al. 1982; Rohwer 1984a, b). Furthermore, studies using ionizing radiation (Rohwer et al. 1984a) have indicated that inactivation rates of the scrapie agent are similar to those for several small ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses.

The causative agent of TSEs resembles a virus in that it exists as different strains and results in an infectious, transmissible disease. Furthermore, if the agent is a virus, then the existence of the various agent strains, which have distinctive biological and pathological properties (Bruce and Fraser 1991; Kascsak et al. 1991), depends on the presence of a strain-specific nucleic acid. However, a specific nucleic acid that copurifies with infectivity has not yet been identified (Duguid et al. 1988; Manuelidis and Manuelidis 1981; Oesch et al. 1988; Sklaviadis et al. 1993). Circumstantial evidence suggesting the absence of a nucleic acid is that the infectious agent is resistant to treatments, e.g., nucleases, psoralens, beta-propiolactone, that typically inactivate or degrade nucleic acids (Bellinger-Kawahara et al. 1987a, b; McKinley et al. 1983). Some papovaviruses, enteroviruses, and hepadnaviruses, however, are also resistant to inactivation by these treatments.

The inability to observe virus particles by electron microscopy in infected tissue (Bots et al. 1971; Cho and Greig 1975; Narang 1974, 1990) has been used to argue against the virus theory. More recent studies, however, have reported the presence of particles resembling virus structures in scrapie-infected hamster brain (Ozel and Diringer 1994. These particles are 10 to 12 nanometers (nm) in diameter, which is smaller than any known virus.

The lack of an immune response in infected tissue should not be used to dispute the virus theory. The immune response to the infection cannot be measured in the absence of well-defined viral-specific antigens, which have not been found in TSEs. It recently has been argued that the astrocyte hypertrophy and microglial activation present in scrapie-affected mice represent a modified inflammatory response (Williams et al. 1994). Induction of cytokines, prostaglandin, and lipocortin-1 also may contribute to this immune reaction (Williams et al. 1997).

Additional evidence that argues against the virus theory is the copurification of scrapie infectivity with the scrapie protein (PrP^{Sc}) (Bolton et al. 1982; Diringer et al. 1983; Safar et al. 1990). These fractions, however, also contain low amounts of nucleic acid (Meyer et al. 1991). Furthermore, under certain conditions, the infectivity can be dissociated from PrP^{Sc} (Czub et al. 1986, 1988; Manuelidis et al. 1987; Xi et al. 1992).

Taken together, these results indicate that the virus cannot be ruled out and that the infectious agent associated with TSEs could be an unconventional virus.

Virino Hypothesis

The virino theory, which proposes that the infectious agent is a small, noncoding nucleic acid surrounded and protected by a host-coded protein, was first described by Dickinson and Outram (1979). The lack of an immune response and the existence of different scrapie strains were the major factors contributing to the virino theory. Despite numerous attempts (Bellinger-Kawahara et al. 1987a, b; Borras and Gibbs 1986; Duguid et al. 1988; Wietgrefe 1985), the nucleic acid component of the virino has not been identified. This inability may be because of its small size or its similarity with contaminating host nucleic acids. Furthermore, proponents of the virino theory suggest that the unconventional nature of the infectious agent is

because the nucleic acid is so well protected by its association with host proteins.

Modified Host Protein (Prion) Hypothesis

According to this hypothesis, the infectious agent is composed solely of an aberrantly processed (probably through a post-translational event) host-coded protein. This abnormal protein is thus rendered infectious and can catalyze its own accumulation. A likely candidate for this protein is PrP.

Although a number of studies have implicated PrP^{Sc} as being responsible for neuropathology in vivo, expression of PrP^{c} in the target tissue is required (Brandner et al. 1996). Furthermore, in vitro studies using pulse-chase metabolic labeling have shown that the conversion of PrP^{c} to PrP^{Sc} probably occurs in a cholesterol-containing plasma membrane compartment or in the endocytic pathway to the lysosomes (Caughey et al. 1991; Taraboulos et al. 1995). Upon infection, the predominantly α-helical conformation of PrP^{c} is post-translationally modified and converted into the β-pleated sheet structure of PrP^{Sc}. However, the mechanism responsible for the conversion of PrP^{c} to PrP^{Sc} is not fully understood. Conversion seems to involve a direct interaction between the two proteins. Studies using transgenic mice (Prusiner et al. 1990; Scott et al. 1992), cell culture (Priola et al. 1994; Priola and Chesebro 1995), and cell-free reactions (Kocisko et al. 1994, 1995; Raymond et al. 1997) clearly have shown that amino acid sequence homology between PrP^{c} of the host and PrP^{Sc} from the incoming infectious agent is required for efficient PrP^{Sc} formation.

The necessity for species specificity in the PrP cell-free conversion studies resembles the species barrier phenomena known to exist when the infectious agent from one species is used to infect another. This resistance to infection of the new host is because of the sequence dissimilarities between the two forms of prion proteins. Cell-free conversion studies may help define the in vivo mechanisms involved in species susceptibility to TSEs. In addition, if the cell-free conversion of PrP^{c} to PrP^{Sc} results in increased infectivity, this conversion would provide strong supporting evidence for the prion theory.

The explanation for the existence of agent strains has been difficult to reconcile by supporters of the prion theory. Proponents of the virus and virino theories believe that the presence of an agent-specific nucleic acid is responsible for the various agent strain characteristics. However, the prion theory needs to explain the strains in the absence of a nucleic acid component. A recent study using differential antibody binding (Safar et al. 1998) has suggested that the characteristics of each agent strain are controlled by the PrP conformation. This study argues the controversial view that the PrP^{Sc} derived from each strain has a unique beta-pleated sheet conformation. Upon infection, the PrP^{Sc} interacts with alpha-helical PrP^{c} molecules, causing them to assume the strain-specific beta-pleated sheet conformation.

Identification of the etiologic agent responsible for TSEs continues to elude investigators. It is imperative that one maintains an objective approach and considers all the possibilities when interpreting the data. Many intriguing questions remain unanswered concerning this mysterious group of diseases. Elucidation of the nature and etiology of the causative agent should be a research priority.

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