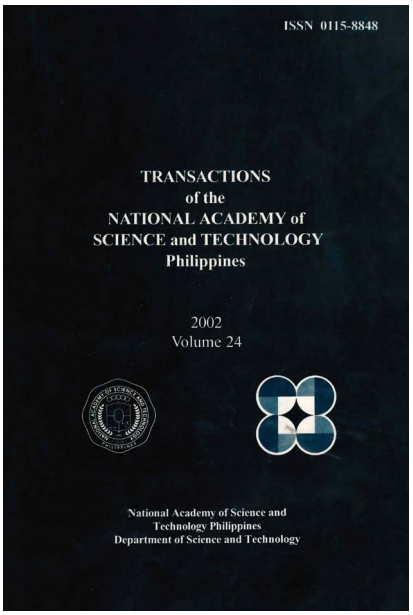
TRANSACTIONSNASTPHL
ISSN 0115-8848 (print)
ISSN 2815-2042 (online)
https://transactions.nast.ph
Vol. 24 Issue No. 2 (2002)
doi.org/10.57043/transnastphl.2002.5088



Prion Diseases in Animals

Acd. Salcedo L. Eduardo

Member, National Academy of Science and Technology Philippines
Dean and Professor, College of Veterinary Medicine
University of the Philippines Los Baños
College, 4031 Laguna, Philippines

Citation
Eduardo SL. 2002. Prion diseases in animals. Transactions NAST PHL 24(2): 159-170. doi.org/10.57043/transnastphl.2002.5088

Trans. Natl. Acad. Sci. Tech. Philippines 24: 159-170 (2002).
ISSN 0115-8848

PRION DISEASES IN ANIMALS

SALCEDO L. EDUARDO
Member, National Academy of Science and Technology Philippines
Dean and Professor, College of Veterinary Medicine
University of the Philippines Los Baños
College, 4031 Laguna, Philippines

ABSTRACT

Prion diseases refer to a group of invariably neurodegenerative diseases in human and animals also known collectively as transmissible spongiform encephalopathies (TSE). These are caused by proteinaceous infectious particles that lack nucleic acid called prions. This paper is a review of prions which occur in animals and human spongiform encephalopathies are excluded.

Prions occurring in animals include scrapie or ovine spongiform encephalopathy, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy (TME), chronic wasting disease (CWD) of mule deer and elk, and feline spongiform encephalopathy (FSE). These diseases share many characteristics of a long incubation period (from months to years or decades), a clinical course also lasting from weeks to years resulting to death, lesions which are, for the most part, restricted to the central nervous system and the lack of immune response. Changes include neuronal degeneration with neuronal vacuolation (spongiform degeneration), reactive astrocytosis and often "amyloid plaque" formation. Variations exist for several of the spongiform encephalopathies as to disease incidence, breed and species susceptibility and incubation time. Except for scrapie and BSE, information on the other animal spongiform encephalopathies is wanting. The occurrence, host range, signs, histopathology, transmission and diagnosis especially for scrapie and BSE are presented and discussed. It has been suggested that BSE has resulted from ingestion by cattle of meat and bone from scrapie-infected sheep. TME is also considered to have resulted by the same route. It is also believed that bovine to bovine transmission results from feeding with bovine meat ans bone meal. Thus, the ban on the use of meat and bone meal from ruminants has reduced the occurrence of BSE.

There is no direct evidence that any of the animal spongiform encephalopathies is transmissible to humans. However, cases of a new variant Creutzfeld-Jacob Disease (vCJD) which occurred in teenagers and young adults in Britain and France revealed lesions of neurologic changes not previously seen in CJD cases of adults in the United States, Australia or Japan. These changes (numerous amyloid plaques) are similar to those seen in macaques inoculated with bovine prions.

None of the transmissible spongiform encephalopathies herein presented has been reported in the Philippines.

Keywords: BSE, CWD, FSE, prion, prion diseases, scrapie, TME

INTRODUCTION

Prion diseases refer to a group of invariably neurodegenerative diseases in human and animals also known collectively as transmissible spongiform encephalopathies (TSE). These are caused by proteinaceous infectious particles that lack nucleic acid called prions. Since the BSE epidemic and its experimental transmission to other species and the resulting heightened awareness of this disease, TSEs have been the subject of studies for the last two decades. This paper is a review of TSEs occurring only in animals and those in humans are excluded.

THE ETIOLOGIC AGENT CALLED PRION

Some of the TSEs have existed long before the exact nature of the causative agent became known. They have been recognized for their clinical signs and the lesions that they produced. Scrapie of sheep (ovine spongiform encephalopathy) has been known in Scotland for two centuries. Missionaries have known kuru of the Fore people of New Guinea, which is transmitted by ritualistic cannibalism. Transmissible mink encephalopathy was first recognized in 1947 in Wisconsin, USA, chronic wasting disease of captive mule deer in 1978 in Colorado (Jones et al., 1997), BSE in the mid 80s and feline spongiform encephalopathy in 1990 in England (Fraser, 2000).

The etiologic agent of these diseases previously had not been fully understood. Thus several agents have been claimed as follows: Sarcosporidia parasite, filterable virus, small DNA virus, slow virus, DNA subvirus, replicating abnormal polysaccharide, piroplasma-like organism and naked nucleic acid similar to plant viroids (Prusiner, 1982; 1998).

The causative agent is now known to be protein representing an abnormal isoform of a normal protein. Prion is the term proposed by Prusiner (1982) to be the cause of these transmissible spongiform encephalopathies. He defined prions as proteineaceous infectious particles that lack nucleic acid and are composed of

abnormal pathogenic isoform (PrP^{sc}) of a normal cellular protein (PrP^{c}). For his discovery of prions and for elucidating the principles that underlie their mode of action, he was awarded the 1997 Nobel Prize in Physiology and Medicine. Prion is resistant to ultraviolet and ionizing radiation, ultrasonification, proteases and nucleases, heat (even beyond 100°C, for one hour) and most chemicals. It is incompletely inactivated at 100°C and extra resistant to 300°C in a dry state. Wet heat is far more effective than dry heat. It has extraordinary resistance if heated to over 300°C in a desiccated state.

Prolonged exposure to 10% formalin produces little or no loss of infection or even protects the agent. It is, however, susceptible to sodium hypochlorite, chlorine and sodium hydroxide solution. The first is effective for routine use, sterilizing equipment and cleaning-up regime for non-porous surfaces. Exposure to chlorine at 20,000 ppm will inactivate the agent The latter is completely effective at 1M (4%) for one hour of exposure and 1M for 30 minutes of autoclaving at 121°C.

PRION DISEASES OR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE)

This is a group of diseases in humans and animals which are neurodegenerative and invariably fatal (Fraser, 2000). Members of this group share common features as follows: 1) long incubation period (months to years or decades); 2) clinical course lasts for weeks or years and progressing to death; 3) lesions are restricted in the central nervous system (CNS) which include neuronal degeneration with neuronal vacuolation (spongiform degeneration), reactive astrocytosis and often amyloid plaque formation; and 4) the course and duration of the disease is not altered by immunosuppression or immunopotentiation. These diseases do not therefore elicit immune response.

The diseases in animals that belong to this group include scrapie or ovine spongiform encephalopathy in sheep and goats, bovine spongiform encephalopathy (BSE) or also known as "mad cow disease", chronic wasting disease (CWD) of deer, mule and elk, feline spongiform encephalopathy (FSE) and transmissible mink encephalopathy (TME) and these are discussed below. Those occurring in humans include kuru, Creutzfeld-Jakob disease (CJD)(sporadic, iatrogenic or familial) and its variant, vCJD and Gertsmann-Straussler Syndrome (GSS).

1. Scrapie or Ovine Spongiform Encephalopathy

Scrapie is the common name and is based on the main manifestation, which is continuous scraping (rubbing) of skin against stationary objects because of the intense pruritus the animal has to endure.

It has been known in Scotland for at least two centuries and now recognized throughout Britain, Europe, America and Asia (Jones et al, 1997; Fraser, 2000).

Scrapie was present in Australia, New Zealand and South Africa but strict slaughter programs have eradicated the disease. Scrapie was the first of the prion spongiform encephalopathies shown to be transmissible. Natural infection with scrapie occurred in sheep, goat and mouflon. It is believed that scrapie prion can be transmitted to mice and other species and has crossed species for some time to cause spongiform encephalopathies of other animals and humans. It occurs in adults of both sexes in sheep 2-5 years old.

Ovine strains of scrapie occurring in North America (particularly in the USA)) differ from strains which occur in Europe and were present at the onset of development of TSE in three species of deer living in free nature and in captivity in the USA. Domesticated sheep are the decisive reservoir species of animal TSE. They have been infected to an unknown extent with the causative agent of BSE probably through contaminated meat-bone meal. The occurrence of natural ovine prion isolates with properties similar to of the BSE agent required that scrapie should be included in the surveillance of human and animal TSE. At present, scrapie is a notifiable disease listed in the International Epizootics Office (Novak et al., 2000).

Signs of scrapie include initially as behavioral changes like locomotor incoordination, tremor and hypersensitivity to noise, touch or movement, intense itching with rubbing against objects and terminally quadriplegic, and subsequently as progressive nervous alterations. The intense pruritus causes the sheep to rub against objects continuously until the wool coat is almost completely lost. Animal may fall into epileftiform seizure of brief duration if startled. Clinical phase can last several weeks or longer. The animal usually grinds its teeth and the voice may be altered. Sometimes it can be rapid (a day or so) thus can cause unanticipated death in apparently healthy sheep and goats (Fraser, 2000; Jones et al., 1997). In some cases, amyloid plaques consisting of prion proteins are found in the molecular and granular layers of the cerebellum. There is never recovery from the disease. There are no characteristic lesions in this disease. The specific tissue alterations are limited to microscopic changes in the medulla oblongata, pons, midbrain and spinal cord. The presence of vacuoles in the cytoplasm of neurons associated with rather diffuse astrogliosis and accumulation of lymphocytes are diagnostic of the infection. Lesions are numerous in the nuclei of the medulla and are not found in the cerebral cortex or cerebellum.

It is transmitted by contact from affected or infected healthy sheep to uninfected sheep and goats. Sheep placenta may be one source of direct infection and pasture contamination. Infected placenta may be eaten or may contaminate pasture (horizontal transmission). Infected feces may also contaminate pasture. Ecto- and endo-parasites might act as reservoirs of scrapie. It is frequently transmitted by family lines where progeny of sheep that develop scrapie are more likely to get the disease, than those from apparently scrapie-free sheep. Maternal transmission is recognized as important in natural sheep scrapie. Sheep derived from unwashed enbryos develop scrapie in Embryo Transfer (Fraser, 2000). Scrapie

has also been experimentally transmitted to sheep by oral, nasal and parenteral routes. Brain extracts from sheep infected with scrapie fed to cattle produced illness substantially different from BSE.

The epidemiology of scrapie in goats is not fully understood. Few cases have been reported since the first case in France in 1942. Infection may pass from sheep to goats or between goats in a similar way to that between sheep implying that both vertical and horizontal transmission can occur in goats (Fraser, 2000).

2. Bovine Spongiform Encephalopathy (BSE) or "Madcow Disease"

"Mad cow disease" according to Hamilton (2001) is an inaccurate term used to describe Bovine Spongiform Encephalopathy (BSE), because cows to not appear "mad" or "crazy" when they have the disease. This is a term coined by the news media in order to gain public attention and sensationalize the story. BSE is the more appropriate term. This disease was first recognized in England in cattle in the mid 1980s (Wilesmith et al., 1988) and became an important disease of cattle in the U.K. About 180,000 cases of BSE were confirmed in the U.K. between November 1986 and December 2000. Cases of BSE outside the U.K. were reported from 1989 in relatively small numbers (a total of about 1,300) in native cattle in Europe (Belgium, Denmark, France, Ireland, Liechtenstein, Luxembourg, Netherlands, Portugal and Switzerland, Germany and Spain) and Asia (Japan, Korea, Thailand). Small number of cases have also been reported in Canada, the Falkland Islands, Italy and Oman but solely in animals imported from the U.K.

Affected animals develop slowly progressive behavior and locomotion changes. They become excitable and exhibit apprehension and aggression when approached, handled and disturbed. Ataxia of the hindlegs occurs causing the animals to stumble and fall. These signs along with weakness and loss of good bodily condition progress over 1 to 4 months, leading to death or slaughter. The disorder has been seen in dairy cows between 2 and 5 years of age (Jones et al., 1997)

Lesions are confined to the nervous system and resemble the other subacute spongiform encephalopathies and vacuolation of neurons and neuropils, leading to a spongiform appearance of the gray matter and are most pronounced in brain stem nuclei. There is a mild gliosis. Amyloid plaques have not been described, but scrapie-associated fibrils have been recovered from affected brains. In cattle, the BSE agent is found outside the CNS only in the Peyer's patches (Wells et al., 1987; Fraser, 2000)

In pigs inoculated with brain homogenates of naturally BSE cases, lesions typical of BSE developed which consisted principally of severe neuropil vacuolation affecting most areas of the brain, but mainly the forebrain (Ryder et al., 2000).
BSE has been transmitted experimentally to mice, pigs, sheep, goats and marmosets through intracerebral inoculation of BSE infected cattle brain extracts. Mink experimentally infected with BSE produced encephalopathy with minimal

resemblance to TME (Fraser, 2000). Direct spread of BSE between cattle has not been documented. BSE however has been transferred to cattle through parenteral inoculation of brain homogenates. Macaques and marmosets inoculated with bovine prions developed neurologic disease several years after inoculation, but only the former exhibited numerous PrP plaques similar to those found in vCJD.

Rodent experiment showed the lympho-reticular system as the main site of replication and that the agent reaches the CNS via the peripheral autonomic, sympathetic nervous system which is known to innervate the lymphoid system. In sheep, scrapie and BSE reach high levels in lymphoid tissue such as spleen (Fraser, 2000)

In natural cases of BSE in cattle, infectivity has been found only in the CNS (the brain, the spinal cord and retina). No infectivity has been found in about 50 tissues including bone marrow, clotted blood, buffy coat, serum or fetal calf serum, or in pathogenesis study in blood or assayed component of blood (Bradley, 1999).

Epidemiological analysis revealed the source of the agent as meat and bone meal used in supplementary feeding of cattle starting in 1981. This was the result of changes in manufacturing process. The epidemic was amplified by recycling infected ruminant tissue, and most cattle became infected as calves. There is little evidence of spread between cattle or to other species other than ruminant protein used in feed (Fraser, 2000).

3. Transmissible Mink Encephalopathy (TME)

This disease is rare and occurs in farmed mink with very high mortality, reaching 100%. It has been reported in North America, Russia and Finland. Clinical signs of the disease include a slowly progressive locomotor incoordination, excitability, late somnolence, and, occasionally, convulsions. Death follows a course of 3 to 8 weeks. Lesions are restricted to the central nervous system, where widespread neuronal degeneration and marked astrogliosis are found in the cerebrum, cerebellum and brainstem. Neurons, especially in the cerebellar peduncles, may contain cytoplasmic vacuoles similar to those seen in scrapie (Jones et al., 1997)

The source of the agent is the feed. Spread between mink is very limited, as there is no vertical or maternal transmission. Infection may however be transmitted through wounds during fighting and cannibalism. TME prion is present only at low levels in spleens or none at all. It is considered to have the same properties as those causing scrapie and BSE (Fraser, 2000).

It has been demonstrated that mink brain homogenates inoculated to calves produced encephalopathy 18 months later. It is believed that TME arises from feeding of affected sheep parts, subsequently spread among mink through fighting and cannibalism. TME outbreak in England occurred at the same time as BSE. However, TME outbreak in the US occurred in the absence of feeding affected sheep parts.

4. Feline Spongiform Encephalopathy (FSE)

This disease was first described in cats in Britain in 1990 and several cases have been recorded since then. Cases have been diagnosed in Britain, Northern Ireland, Norway and Luxembourg. This disease is epidemiologically similar to TME, both arising from a herbivorous species in their carnivorous diet (Fraser, 2000).

Affected animals show various neurologic signs such as behavioral changes, head and hindquarter tremors, ungainly gait, and marked ataxia. Other clinical signs include dilated pupil that is unresponsive to light, later profuse salivation, hyperaesthesia especially to sound and hypermetria but no pruritus (Leggett et al., 1990, Jones et al., 1997).

Lesions are confined to the brain, which include vacuolation of the neuropil and vacuoles in the cell bodies of neurons at all levels. These are more pronounced in the nuclei of the central grey matter around the mesencephalic aqueduct and some extending to the axonal process. There are no plaques of amyloid materials (Leggett et al., 1990).

5. Chronic Wasting Disease (CWD) of Deer

The disease occurs in 3-4-year old deer in most cases. It was first recognized in mule deer in the late 1960s in Wyoming and Colorado, USA. Recent reports on other animals infected includes black-tailed deer, white-tailed x mule deer and Rocky Mountain elk.

The disease is characterized by emaciation, changes in behavior and excessive salivation in both species and deer, and also polydipsia and polyuria. Clinical course is from several weeks to 8 months and the disease is invariably fatal. At necropsy, animals are emaciated but other gross lesions have not been found consistently. Ruminal contents of deer are excessively fluid, reflecting polydypsia in this species. Various other gross changes, particularly aspiration pneumonia, are inconsistently present and have been considered the result of intercurrent or secondary disease processes. Consistent microscopic changes of vacuolation (spongiform) have been found only in the central nervous system (Williams and Young, 1993; Jones et al., 1997).

Although successful oral transmission has been achieved to mule deer fawns using brain homogenate of naturally infected mule deer with CWD, there is no evidence for a dietary source of the infection as no animal protein is fed, other than milk and milk products to fawns. Sheep, cattle and goats in the same area with the infected deer showed no evidence of infection. However, goats, mink, ferrets and squirrel monkey were experimentally infected with the disease (Fraser, 2000).

DIAGNOSIS

Diagnosis of TSEs is made by a combination of clinical signs and pathologic findings at postmortem. Histo-pathological examination of the brain is conducted to reveal characteristic spongy degeneration and vacuolation of nerve cells and sometimes, amyloid plaques (Jones et al., 1997). The presence of abnormal fibrils can be demonstrated by electron microscopy.

Immuno-histochemistry is employed to detect the abnormal, disease-specific, protease-resistant form of PrP. The paraffin-embeded tissue (PET) blot is used to detect prion PrP^{sc} deposits in formalin-fixed and paraffin-embeded tissue (Schulz-Schaeffer et al., 2000). Immunoblotting is employed for the detection of proteinase K-resistant prion protein in BSE (Madec et al., 2000) and even brain and tissue extracts prepared with detergents. Western immunoblotting procedure for bovine PrP^{sc} detection has proved useful as a rapid surveillance method to identify animals subclinically infected with BSE (Schaller et al., 1999). Oesch et al. (2000) have shown that prionics western blotting procedure is useful in screening for BSE in cattle regularly at slaughter abattoirs in Switzerland. This method has allowed rapid analysis of hundreds of samples per day on a routine basis without causing delays to the meat processing industry. It has also been employed for the diagnosis of BSE in the UK (Cooley et al., 2001) and in Korea (Koo et al., 2001).

Sigurdson et al. (1999) similarly employed a modified immuno-histochemistry assay that enhanced sensitivity and detected abnormal prion isoform (PrP^{res}) of CWD in alimentary tract associated lymphoid tissues such as retropharyngeal lymph node, tonsil, Peyer patch and ileocecal lymph nodes, as early as 42 days p.i. thereafter, 53 to 80 days.

The same immuno-histochemical methods can be employed in the identification of infection in healthy and pre-clinically infected animals. Its use however is limited because the abnormal prion isoform is present only in very few tissues other than the brain. This limits the kind of tissue that can be taken at biopsy. Thus, only lymphoid tissues (tonsils, third eyelid or peripheral lymph nodes) for scrapie (Roels et al., 1999; Koo et al., 2001), lymphoid tissues associated with the alimentary tract and tonsils for CWD (Sigurdson et al, 1999) and Peyer's patch at the distal ileum for BSE (Fraser, 2000).

A model for predicting the number of cases of vCJD in the human population in the UK as a result of the BSE epidemic has been formulated (Ghani et al., 2000) This model estimates that the highest number affected will be 136,000. The model utilized data of vCDJ up to 2001 and assumed that only persons having two copies of the methionine codon at codon 129 of the PrP gene are susceptible. The model also indicates that, on the average, no more than two cases of cVJD could arise from one maximally infected bovine entering the food chain.

No test is available for detecting infection of carrier animals or those incubating the disease.

PREVENTION AND CONTROL

Although there is no treatment for any of the transmissible spongiform encephalopathies herein presented, there are practical methods of controlling their spread.

Scrapie has been eradicated from Australia and New Zealand by compulsory slaughter of imported sheep or flockmates. It can be controlled and the incidence reduced by reducing infectious load through good husbandry and selecting for resistance (genetic control). The latter is a new and major opportunity of controlling the disease using PrP-genotyping to determine susceptibility to the infection. The age when the disease occurs depends on incubation period, which is genetically controlled, age at initial infection and infectious dose (Fraser, 2000). Tests are now available commercially to determine the PrP genotype of sheep.

Targeted surveillance which involves identifying and testing high-risk populations is essential for assessing the regional or country-specific status for disease such as BSE where the number of infected animals in the general population might be low (Doherr et al., 1999)

Prohibition of ruminant derived protein in ruminant rations has reduced BSE in other countries.

It is recommended that extreme caution should be observed in handling animals infected with or suspected of TSE. Animal should be disposed of by incineration. Animal tissues and other materials should be soaked in 2N sodium hydroxide solution for at least 1 hour, autoclaved and incinerated. Instruments and contaminated areas should be decontaminated using the above solution and the former, rinsed in water before autoclaving. Detailed laboratory procedures can be obtained from the National Institutes of Health, Bethesda, Maryland, USA.

WHO (2000) has made recommendations to reduce exposure to the BSE agent and these are given below:

1. Prohibit the use of ruminant tissues in ruminant feed and must exclude tissues that are likely to contain the BSE agent from any animal or human food chain.
2. Countries are encouraged to conduct risk assessment to determine if they are at risk for BSE in sheep and goats.
3. Milk and milk products are considered safe. Tallow and gelatin are considered as safe if prepared by manufacturing process, which has been shown experimentally to inactivate the transmissible agent.
4. Human and veterinary vaccines prepared from bovine materials may carry the risk of transmission of animal TSE agents. The pharmaceutical industry should ideally avoid the use of bovine materials and materials from other animal species in which TSEs naturally occur. If absolutely necessary, bovine materials should be obtained from countries which have surveillance system for BSE in place and which report either zero or only sporadic cases of BSE. These precautions apply to the manufacture of cosmetics as well.

In the Philippines, none of the transmissible spongiform encephalopathies herein presented has been reported. Measures have been taken to prevent their entrance to the country. Thus, the Secretary of Agriculture of the Philippines has issued Memorandum Order No. 8, series of 2001 "Temporary Ban on the Importation of Meat and Bone Meal from all Countries." Its implementing guidelines are as follows:

- The temporary ban includes meat and bone meal (MBM), meat meal and bone meal which are used as ingredients for animal feeds.
- Meat and bone meal (MBM), meat meal and bone meal not covered by the ban are those coming from BSE-free countries that were shipped from the country of origin on or before July 18, 2001, as shown in the Bill of Lading, provided that there is an International Veterinary Certificate attesting that these came from animals that are healthy. As a condition for release of these shipments, the importers must certify that said products should be distributed only to poultry and piggery farms. After the actual usage, the importer shall submit a report on the actual distribution of these imported feed ingredients.

ECONOMIC IMPACT

The impact of BSE on a regional perspective has been studied by Caskie et al. (1999) in Northern Ireland, a region heavily dependent on beef export using a regional input-output model. The model estimated the long run regional output, income and employment effects. It assumed no market stabilization measures and took into account substitution effects in final demand. The predicted net loss in regional income was 0.5% of regional GDP with job losses of up to 0.6% of regional employment. Seventy seven percent of the income losses and 87% of the job losses are in the beef sectors, primarily beef production. Compensating gains due to demand substitution effects occurred mainly in meat processing sectors, other than beef, and were relatively small.

REFERENCES

Bradley, R. 1999. BSE transmission studies with particular reference to blood. In: Animal Sera, Animal Sera Derivatives and Substitutes Used in the Manufacture of Pharmaceutical: Viral Safety and Regulatory Aspects (Brown, F., T. Cartwright, F. Horaud and J.M. Spieser, editors), S. Karger AG, Basel, Switzerland, pp. 35-40.

Caskie, P., J. Davies, and J.E. Moss. 1999. The economic impact of BSE: a regional perspective. Applied Economics 31(12):623-1630.

Cooley, W.A., J.K. Clark, S.J. Ryder, L.A. Davis, S.S.J. Farrelly and M.J. Stack. 2001. Evaluation of rapid Western immunoblotting procedure for the diagnosis of bovine spongiform encephalopathy (BSE) in the UK. J. Comparative Pathology 125(1):64-70.

Doherr, M.G., B. Oesch, M. Moser, M. Vandevelde and D. Heim. 1999. Targeted surveillance for bovine spongiform encephalopathy. Veterinary Record 145(23):672.

Fraser, H. 2000. Scrapie in sheep and goats and related diseases. In: Diseases of Sheep. Third Edition (Martin, W.B. and I.D. Aitken, editors), Blackwell Science, London, pp. 207-218.

Ghani, A.C., N.M. Ferguson, C.A. Donnelly and R.M. Anderson. 2000. Predicted vCJD mortality in Great Britain, Modelling the latest data puts a ceiling on the likely number of vCJD cases. Nature (London) 406 (6796):583-584.

Hamilton, C.R. 2001. Rendered products of United States origin – continue to be safe to use. Technical Report, Darling International Inc., 7 pp (loose leaves).

Jones, T.C., D.H. Hunt, and N.W. King. 1997. Veterinary Pathology, Sixth Edition. Williams & Wilkins, Baltimore.

Koo, H-C, Y-H. Park, B-C. Lee, C-H. Chae, K.I. O'Rouke and T.V. Basler. 2001. Immunohistochemical detection of prion protein (PrP^{sc}) and epidemiological study of BSE in Korea. J. of Veterinary Science 2(1):25-31.

Leggett, M.M., J. Dukes and H.M. Pirie, 1990. A spongiform encephalopathy in a cat. Veterinary Record 126:586-588.

Madec, J.Y., P. Belli, D. Calava and Th. Baron. 2000. Efficiency of Western blotting for the specific immunodetection of proteinase K-resistant prion protein in BSE diagnosis in France. Veterinary Record 146(3):74-76.

Novak, M., O.J. Vrtiak, I. Mikula and L. Tkacikova. 2000. Ovine scrapie: Priorities and importance (Review). Folia Microbiologica 45(6):475-483.

Oesch, B., M. Doherr, D. Heim, K. Fischer, S. Egli, S. Bolliger, K. Biffiger, O. Schaller, M. Vandevelde and M. Moser. 2000. Application of prionics western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs. In: Prion Diseases: Diagnosis and Pathogenesis (Groschup, M.H. and H. Kretzschmar: editors), Springer Verlag, Wien, Austria, pp. 189-195.

Prusiner, S.B. 1982. Novel proteinaceous infectious particles case scrapie. Science, 216 (4542):136-144.

Prusiner, S.B. 1998. Prions. Proc. Natl. Acad. Sci., USA 95: 3363-13383.

Roels, S., E.Vanopdenbosch, J.P.M. Langeveld and B.E.C. Schreuder. 1999. Immunochemical evaluation of tonsillar tissue for preclinical screening of scrapie base on surveillance in Belgium. Veterinary Record 145(18):534-525.

Ryder, S.J., S.A.C. Hawkins, M. Dawson and G.A.H. Wells. 2000. The neuropathology of experimental bovine spongiform encephalopathy in the pig. J. Comparative Pathology 122(2/3):131-143.

Schaller, O., R. Fatzer, M. Stack, J. Clark, W. Cooley, K. Biffiger, S. Egli, M. Doherr, M. Vandevelde, D. Heim, B. Oesch, and M. Moser. 1999. Validation of a western immunoblotting procedure for bovine PrP^{sc} detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE). Acta Neuropathologica 98 (5):437-443.

Schulz-Schaeffer, W.J., R. Fatzer, M. Vandevelde and H.A. Kretzschmar. 2000. Detection of PrPsc in subclinical BSE with the paraffin-embedded tissue (PET) blot. In: Prion Diseases: Diagnosis and Pathogenesis (Groschup, M.H. and H. Kretzschmar, editors), Springer Verlag, Wien, Austria, pp. 173-180.

Sigurdson, C.J., E.S. William, M.W. Miller, T.R. Spraker, K.I. O'Rourke and E.A. Hoover. 1999. Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (Odocoileus hemionus). J. General Virology 80(10):2757-2764.

Wells, G.A.H., A.C. Scott, C.T. Johnson, R.F. Gunning, R.D.Hancock, M. Jeffrey, M. Dawson and R. Bradley. 1987. A novel progressive spongiform encephalopathy in cattle. Veterinary Record 118:419-420.

WHO . 2000. Press release, Fact Sheets and Features. Fact sheet No. 113, Revised December, 2000. WHO web site: www.who.int

Williams, E.S. and S. Young. 1993. Neuropathology of chronic wasting disease of mule deer (Odocoileus hemionus) and elk (Cervus elaphus nelsoni). Veterinary Pathology 30:36-45.

Wilesmith, J.W., G.A.H. Wells and M.P. Cranwell. 1988. Bovine spongiform encephalopathy: epidemiological studies. Veterinary Record 123:638-644.