Other animal prion diseases

Christina J Sigurdson* and **Michael W Miller†**

**Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, USA and †Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, Colorado, USA*

In addition to bovine spongiform encephalopathy (BSE) of cattle and scrapie of sheep and goats, a few other animal prion diseases have been reported. These include feline spongiform encephalopathy of zoological and domestic cats (FSE) and transmissible spongiform encephalopathy (TSE) of zoological ruminants and non-human primates, as well as chronic wasting disease of deer and elk (CWD) and transmissible mink encephalopathy of farmed mink (TME). The origins of TSE in cats, zoo bovids, and non-human primates are clearly linked to the BSE epidemic; however, the origins of CWD and TME are less clear, but are not epidemiologically linked to the BSE epidemic. Here we review the epidemiology, transmission, clinical features and pathology of these other animal prion diseases.

Spongiform encephalopathy in zoo ruminants, cats, and non-human primates

BSE transmits to non-domestic bovids, felids, and non-human primates

Parallel to the BSE epidemic in cattle beginning in the 1980s, 15 additional species have contracted a spongiform encephalopathy, virtually tripling the number of animal species known world-wide to develop a TSE naturally. Seven bovid, 4 felid, and 4 primate species were afflicted with a TSE, primarily in zoological collections in Great Britain but also in France (Table 1)[1–5]. At the time of their diagnoses, the geographic and temporal association with BSE suggested possible links to the epidemic, and further epidemiological and experimental evidence has bolstered this premise. Affected animals had either consumed cattle-derived protein supplements or were in contact with prion-infected individuals[1,6]. Additionally, mice inoculated with brain homogenates from TSE-infected kudu, nyala, or domestic cats developed a spongiform encephalopathy with profiles of histological lesions and incubation periods virtually identical to those seen with BSE in mice[7,8]. Moreover, the similar biochemical profiles of the protease-resistant prion protein, PrP^{res}, in experimental murine BSE, FSE, and experimental BSE in a macaque supported the hypothesis that these apparently novel TSEs had

Correspondence to: Prof. Christina Sigurdson, Research Fellow, Institute of Neuropathology, University Hospital of Zürich, Schmelzbergstrasse 12, Zürich CH 8091, Switzerland Email: Christina.Sigurdson@usz.ch

British Medical Bulletin 2003; **66**: 199–212
DOI 10.1093/bmb/dg66.199

Table 1 Zoo animals diagnosed with transmissible spongiform encephalopathy between 1985 and 1998[1,3–5]

Species	Number affected
Greater kudu (*Tragelaphus strepsiceros*)	6
Eland (*Taurotragus oryx*)	5
Gemsbok (*Oryx gazella*)	1
Nyala (*Tragelaphus angasi*)	1
Arabian oryx (*Oryx leucoryx*)	1
Scimitar-horned oryx (*Oryx dammah*)	1
Bison (*Bison bison*)	1
Cheetah (*Acinonyx jubatus*)	7
Puma (*Felis concolor*)	3
Ocelot (*Felis pardalis*)	2
Tiger (*Panthera tigris*)	1
Mayotte brown lemur (*Eulemur fulvus mayottensis*)	2
White fronted brown lemur (*Eulemur fulvus albifrons*)	1
Mongoose lemur (*Eulemur mongoz*)	1
Rhesus macaque (*Macaca mulatta*)	1

the same origin – BSE[9]. Thus, the assemblage of epidemiological and biochemical clues has provided compelling evidence that these newly described TSEs arose from BSE that had crossed species barriers.

Although a multitude of zoo species were exposed to BSE-contaminated meat and bone meal, only a small group of animals developed disease. The exotic zoo ruminants that died of TSE include greater kudu, eland, nyala, gemsbok, Arabian oryx, a scimitar-horned oryx, and a bison[1,5]; all are members of the family Bovidae. Most affected animals had consumed diets that included ruminant-derived meat and bone meal. The possible exception was greater kudu. Epidemiological studies initially suggested that kudu developed TSE from exposure to food-borne BSE, but then maintained the infection by horizontal spread among animals in a manner similar to scrapie and CWD[6]; however, the apparently prolonged epidemic may have been the product of sustained exposure to BSE-contaminated feed[5].

Feline spongiform encephalopathy

The prion diseases of non-domestic cats were likely due to ingestion of BSE-infected cattle carcasses. Feline spongiform encephalopathy has been described in a captive cheetah, puma, an ocelot, and a tiger from zoological collections in Great Britain[1,5].

In addition to the non-domestic felids, 87 domestic cats in Great Britain and sporadic cases in Norway, Northern Ireland and Liechtenstein have been diagnosed with FSE[10]. All cats were > 2 years old. Clinically, affected

cats initially demonstrated behaviour changes (more timid or aggressive), with subsequent ataxia, hypermetria, and hyperesthesia to sound and touch[11,12]. Histopathology revealed spongiform degeneration in the neuropil of the brain and spinal cord with the most severe lesions localized to the medial geniculate nucleus of the thalamus and the basal nuclei[10]. A ban on bovine spleen and CNS tissue in pet foods was initiated in 1990, and all but one of the FSE cases to date occurred in cats born prior to the ban[13].

Spongiform encephalopathy in non-human primates

Lemurs and a rhesus macaque from a zoo and three primate facilities in France naturally developed TSE in the 1990s. Primate diets had included meat-meal supplements that were likely contaminated by British beef[4]. Indeed, lemurs experimentally infected with BSE developed brain lesions that were similar to those seen in naturally infected lemurs. Additionally, the immunohistochemical staining patterns in natural and experimental cases were similar and revealed PrP^{res} in tonsil, Peyer's patches, lymph nodes and spleen[4].

Chronic wasting disease: a prion disease in North American deer and elk

Transmission and epidemiology

Chronic wasting disease (CWD) is the only prion disease known to affect free-ranging wild-life. First recognized as a clinical syndrome of captive mule deer (*Odocoileus hemionus*) in Colorado in the 1960s, CWD was not diagnosed as a TSE until 1978, and was diagnosed in captive research deer and captive Rocky Mountain elk (*Cervus elaphus nelsoni*) in southeastern Wyoming soon thereafter[14,15]. Beginning in 1981, cases of CWD were diagnosed in free-ranging mule deer, white-tailed deer (*O. virginanus)* and Rocky Mountain elk (cervids) on the eastern slope of the Rocky Mountains and extending out on the plains following river valleys within Colorado and Wyoming[16,17]. The origin of CWD in captive or free-ranging deer remains enigmatic[17,18].

CWD was first diagnosed in Canada's farmed elk industry in 1996, and in the US elk industry in 1997. More recently, CWD-infected ranched elk have been discovered in several other states and in Canada and South Korea; these discoveries have heightened international awareness and concern regarding CWD and other animal TSEs. Prior to

Fig. 1 The geographical distribution of CWD in free-ranging deer in the US and Canada.

2000, CWD in free-ranging deer was believed to be limited to a focal geographic region of the US. Unfortunately, in the last two years, CWD has been detected in free-ranging deer in Wisconsin, Nebraska, South Dakota, New Mexico, and western Colorado, and in Saskatchewan, Canada (Fig. 1); the origins of these recent outbreaks remain under investigation, but in most cases spill-over from infected game farms seems the most plausible explanation. The appearance of CWD in wild deer presents significant challenges to disease control or eradication due to the extensive geographic range of North American deer and elk, lack of *ante-mortem* diagnostic tests, as well as an inability to rid the environment of potential prion-contaminated excreta[18].

CWD is naturally transmitted with remarkable efficiency. Estimates of CWD-infected deer revealed a prevalence of 1–15% within a defined endemic region in northeastern Colorado and southeastern Wyoming[17]. The efficiency of CWD transmission was also evident in a captive mule deer herd, wherein ~90% of animals (n = 60) resident for 2 years or longer developed CWD between 1970 and 1981[14]. The original source of infection was not determined, but these animals had not been fed

meat and bone meal[19]. The mechanism of CWD agent shedding and natural transmission among free-ranging herbivores is unknown. Epidemiological studies of natural disease suggest horizontal spread potentially *via* the ingestion of forage or water contaminated by infectious secretions, excretions, or other tissue source (*e.g.* placenta or decomposed carcasses), although vertical transmission has not been excluded[17–20]. The abundant presence of the pathogenic prion protein, PrP^{CWD}, in alimentary mucosal-associated lymphoid tissues may favour prion shedding into the environment *via* faeces or saliva[5,18,21].

CWD surveillance within and around the endemic areas of north-eastern Colorado and southeastern Wyoming from 1997 to present has been extensive. Brain samples have been acquired from over 12,000 deer and elk sampled via geographically-focused random surveys and were tested by immunohistochemistry using anti-PrP monoclonal antibodies[17,22]. Brain and lymphoid tissue sections have been examined for PrP^{CWD}. Results of the surveys within the suspected endemic area have revealed that ~5% of mule deer, 2% of white-tailed deer, and < 1% of elk are infected, with wide variation within a species sampled from different subpopulations[17]. Over a 3-year period of sampling, CWD prevalence appeared stable[17]; however, more recent trend analyses suggest prevalence is slowly increasing (MW Miller, unpublished findings). Population models predict that if epidemics continue unmanaged, then mule deer populations would be expected to decline dramatically over a 30–50-year period[17,23]. It is not known whether multiple strains exist.

National surveillance efforts

National programmes for CWD surveillance and management are currently under development in both the US and Canada. The United States Department of Agriculture (USDA) currently encourages CWD screening of all captive ranched elk and deer mortalities. In the absence of national programmes, many states and provinces have developed their own surveillance and certification programmes and have restricted movement of deer or elk across state boundaries[18]. Due to lack of an *ante-mortem* diagnostic assay in elk and deer, captive cervid herds with a documented CWD-positive animal are typically eliminated. Thus far, there have been 25 CWD-positive deer and elk herds detected in the US, 40 in Canada, and at least one in South Korea. The USDA has depopulated 11 known-infected herds, plus additional herds in the endemic areas of Colorado and Nebraska (L Creekmore, USDA, personal communication).

Surveillance for CWD in free-ranging cervids, conducted largely by state and provincial wild-life management agencies, employs a

combination of symptomatically-targeted surveillance and random surveys of harvested animals[17,18]. Surveillance data from free-ranging cervids outside endemic portions of Colorado and Wyoming have been assembled annually by the Southeastern Cooperative Wildlife Disease Study (SCWDS). From 1998 to mid-2002, SCWDS received reports indicating that 14,181 deer and elk had been tested for CWD[24]. In 2002, Wisconsin wild-life officials reported CWD-positive deer had been detected in hunter-harvested animals; shortly thereafter, officials in New Mexico reported a confirmed case in a clinical suspect. Discovery of these wild CWD-infected deer in states far from the original endemic area raised several questions. How had CWD spread to these deer populations? Had scrapie repeatedly jumped the species barrier to cause CWD in geographically distant locations? Is CWD a spontaneously arising disease, caused by a potentially enhanced susceptibility of cervids for prion protein conversion? Is CWD spread by infected ranched deer or elk? Or had CWD-infected deer and elk been illegally translocated from Colorado into other states? Hopefully, on-going experimental and epidemiological investigations will answer some, if not all, of these questions.

Clinical signs

Early signs of CWD in clinically affected deer and elk are extremely subtle and include weight loss, behavioural alterations (such as loss of fear of humans), a lowered head and drooping ears. As clinical disease progresses, more noticeable signs like flaccid hypotonic facial muscles, excessive salivation, regurgitation of ruminal fluid, ruminal atony, and polyuria and polydipsia arise. Individuals may develop aspiration pneumonia in late stage disease[14]. Affected animals are typically > 2 years old (average, 3–5 years), with an equal prevalence seen among males and females (Fig 2). Deer may survive up to 7–8 months after onset of clinical signs[14]; elk may survive even longer[20].

Gross and histological pathology

On gross necropsy examination, end-stage clinical CWD cervids are consistently emaciated with serious atrophy of fat; frothy rumen contents, abomasal ulcers, and aspiration pneumonia are observed with less consistency[14,16]. Characteristic histological lesions are confined to the central nervous system and are similar to the other TSEs, namely: intraneuronal vacuolation, neuronal degeneration and loss, extensive neuropil spongiosis, astrocytic hypertrophy and hyperplasia, and

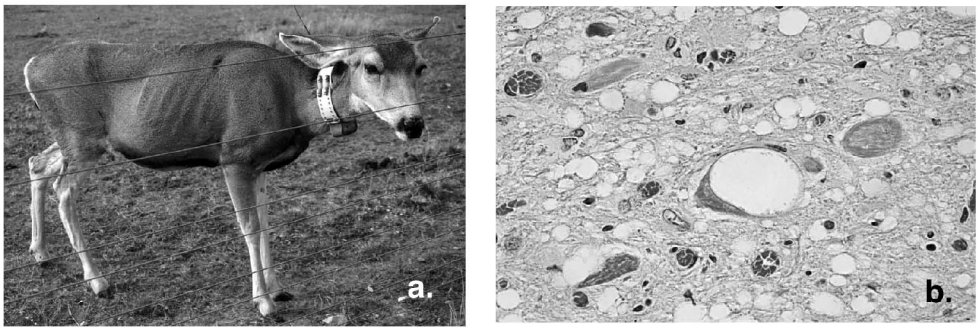

Fig. 2 Deer with clinical CWD; signs are emaciation, depression, weakness, drooping ears, and vacant stare (a). Brain histopathology (b) is characterized by neuronal vacuoles and spongiform degeneration of the neuropil.

occasional amyloid plaques[14,16,19,25]. Spongiform lesions predominate within the thalamus, hypothalamus, midbrain, pons and medulla oblongata as well as in the olfactory tubercle and cortex[14,16]. Severe lesions in the supra-optic and paraventricular nuclei, where anti-diuretic hormone is produced, may be responsible for the clinical signs of polyuria and polydipsia and the low urine specific gravity in clinically dehydrated animals[14].

Interestingly, the distribution of lesions in deer and elk is similar to lesions of BSE in cattle or scrapie in sheep[25] and differs from the lesion distribution of TME, which predominates in the cerebral cortex and basal nuclei[26]. The most consistent histological lesion and PrP^{CWD} immunohistochemical stain of brain is within the dorsal motor nucleus of the vagus nerve[21], which is notably the first site of PrP^{CWD} accumulation (ES Williams and MW Miller, unpublished findings).

Transmission experiments

In the 1980s, Williams and Young demonstrated that CWD was transmissible by intracerebral (IC) inoculation of CWD brain homogenate into deer with an incubation period of 17–21 months[19]. Recently, experiments demonstrated that oral exposure of mule deer fawns to CWD using brain homogenate results in detection of PrP^{CWD} in lymphoid tissues (retropharyngeal lymph node, tonsil, Peyer's patches, ileocaecal lymph node) within 6 weeks' postexposure[27] and clinical disease with an incubation period of 15–25 months (ES Williams and MW Miller, unpublished findings). PrP^{CWD} accumulates within the lymphoid germinal centres in a manner similar to vCJD and scrapie. Phenotyping studies have revealed that within germinal centres, PrP^{CWD} accumulates on cell membranes of follicular dendritic cells and/or B cells

and within the cytoplasm of tangible body macrophages[28]. In advanced cases of CWD in naturally infected deer, PrP^{CWD} accumulates in tonsil, spleen, Peyer's patches, and lymph nodes throughout the body, as well as nerves and ganglia, pancreatic islets, and adrenal medulla[21,29].

Abundance of PrP^{CWD} in lymphoid tissues: implications for diagnosis and transmission

Large accumulations of PrP^{CWD} are detectable by immunohistochemistry in tonsil and other lymphoid tissues of animals affected with CWD[21]. Similar accumulations are also seen in mule deer prior to onset of clinical CWD[22]. Therefore, immunohistochemistry on tonsil biopsies has recently been investigated as a means for *ante-mortem* diagnosis of CWD in deer[30,31]. Interestingly, Kimberlin and others have associated infection of the lymphoreticular system with the transmissibility of scrapie among sheep[32]. It is plausible that the abundant PrP^{res} in alimentary mucosa-associated lymphoid tissues may promote the shedding and efficient transmission of CWD (and scrapie) prions.

Experimental intra- and inter-species transmission of CWD

The natural host range of CWD appears limited to deer (*Odocoileus* spp.) and elk. As with other prion diseases, however, the range of species susceptible to experimental inoculation is somewhat broader. Studies by Marsh and Williams in the mid 1980s demonstrated that the CWD agent could be transmitted to ferrets, mink, squirrel monkeys, and a goat[19]. Bruce and colleagues found mice relatively resistant to CWD infection such that strain typing was problematic[33]. More recently, Bartz and colleagues demonstrated the susceptibility of ferrets to intracerebral inoculation of CWD with an incubation period of 17–21 months[34]. Racoons were reported to be susceptible to scrapie and TME, but not CWD, after intracerebral challenge[35]. As with other TSEs, the susceptibility of other wild-life or domestic species to CWD prions has yet to be studied comprehensively.

Transmission to humans or domestic livestock

Investigations of unusual CJD cases in the US over the last decade have identified no causal relationship with CWD exposure[36], but these investigations, as well as other retrospective and prospective studies of CJD risk factors, are on-going. No higher incidence or unusual clusters of CJD cases have been observed in northeastern Colorado or southeastern Wyoming (J Pape, Colorado Department of Public Health

and Environment, personal communication). Hunting continues as a population management tool within the CWD endemic regions, but public health officials recommend that CWD-infected carcasses not be consumed. The actual risk of CWD jumping the species barrier and causing human disease is unknown. A recent study by Raymond *et al*[37] examined the ability of PrP^{CWD} to convert human PrP^{C} *in vitro* and determined that the conversion was inefficient, but similar to the efficiency of PrP^{BSE} or ovine PrP^{Sc} to convert human PrP^{C}. Because BSE is apparently infectious to humans, at least at a low level, it would seem prudent to limit exposure of humans to CWD.

Could CWD also cross species barriers and infect cattle and sheep sharing grazing areas with CWD-infected deer? CWD transmission to cattle causing a new BSE strain would be economically devastating for the US beef industry. There are several studies, both completed and on-going, to determine whether cattle might be susceptible to CWD:

1 Conversion of bovine PrP^{C} by PrP^{CWD} was relatively inefficient[37] compared to conversions by PrP^{BSE} or ovine PrP^{Sc}.

2 Cattle have been exposed to CWD-infected brain homogenates by the most extreme and unnatural route, intracerebral inoculation. Thus far, at 27 months' post-inoculation (p.i.), 3 of 13 cattle have developed detectable PrP^{res} in brain by Western blot and immunohistochemistry[38].

3 Cattle have been orally inoculated with CWD brain homogenate and show no clinical signs at 62 months' p.i. (ES Williams and MW Miller, unpublished findings).

4 Cattle are living in research facilities among a deer population with a historically high prevalence of CWD and are in close association with deer showing clinical signs of CWD. None of these animals have developed signs of TSE after 63 months' exposure (MW Miller and ES Williams, unpublished findings).

5 A histological and immunohistochemical surveillance of brainstem from 262 cattle over 4 years old grazing in CWD endemic areas has not revealed any suspect lesions of TSE[39].

Thus far, data from these studies indicate that CWD will not readily transmit to cattle.

Genetics

Within the PrP gene of mule deer, white-tailed deer and Rocky Mountain elk, there are three known polymorphisms. Mule deer and white-tailed deer have residues G/S at position 96 and S/N at position 138[37], but no apparent relationship between genotype and CWD susceptibility has

been demonstrated to date in either species. In contrast, the PrP polymorphism in elk occurs at position 132 (M/L), and to date only elk with 132 M/M or M/L have developed disease. In a study by O'Rourke *et al*, elk expressing M/M at residue 132 were significantly over represented among CWD-infected individuals from both free-ranging and captive populations[40], suggesting that the 132 PrP polymorphism (M/L) may influence susceptibility in this species. Evidence of genetic susceptibility to CWD in elk resembles observations in two other host species: Human 129 PrP polymorphism (M/V) has been linked to susceptibility to vCJD and some forms of CJD[41]. Similarly, sheep 136 (V/A) PrP polymorphism has been linked to scrapie susceptibility, as has a second at 171 (Q/R).

In contrast to intensely bred domestic ruminant populations, the PrP genetics of free-ranging cervid populations of different species, subspecies, or breeding populations will require large-scale surveys. Further, since the prevalence of CWD is so low in most populations, estimating relative genetic susceptibility based on field exposure is not likely to be useful, and experimental oral inoculations of animals of various genotypes using an inocula of different genotypes will be necessary (K O'Rourke, personal communication).

Transmissible mink encephalopathy

Epidemiology and transmission – a controversial arena

Transmissible mink encephalopathy (TME), initially recognized in Wisconsin and Minnesota in 1947, has sporadically appeared in farmed mink in several countries where farmed mink are raised, including the US, Canada, Finland, Russia, and East Germany[42]. Nonetheless, TME outbreaks are rare; the most recent occurrence in the US was in 1985. Epidemiological studies of outbreaks indicate that the disease is causally linked to the ingestion of prion-contaminated meat, potentially scrapie sheep[43]. However, in the 1985 TME outbreak in Stetsonville, Wisconsin, the mink rancher stated with certainty that sheep were not fed to mink. Instead, downer (ill) cattle were the primary source of mink food – a discovery which has led to much speculation on a potentially unrecognized BSE-like disease of American cattle[43]. Despite such speculation, the ultimate origins of TME epidemics remain uncertain.

To further investigate potential food-borne sources of TME, mink were intracerebrally (IC) exposed to UK- and North American-derived sheep scrapie brain homogenates. Mink were highly susceptible to the Suffolk sheep scrapie from the US, but only after IC inoculation[44]. Mink did not develop disease from ingesting scrapie brain[45]. These studies suggested, at minimum, that mink are susceptible to scrapie. However,

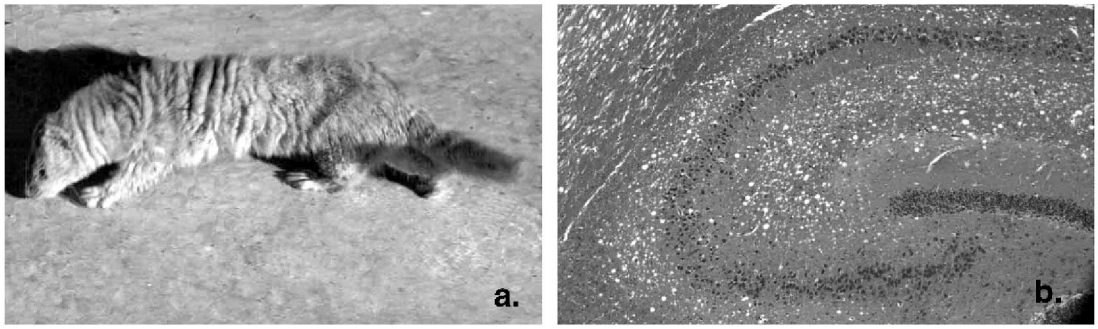

Fig. 3 TME in a mink. Clinical signs of TME include a rough hair coat, extended tail, and ataxia (a). Brain from a mink with TME depicting severe spongiform degeneration in the hippocampus (b). Images were generously provided by Dr Jason Bartz, Creighton University, Nebraska, USA.

further experiments demonstrated that TME could pass into cattle and, moreover, that brain from these cattle could transmit the TME agent efficiently to mink by either the IC or the oral route, with an incubation period of only 4 and 7 months, respectively. This indicates that TSEs can be transmitted efficiently between cattle and mink[46], although the epidemiological significance of these findings are less clear.

Extensive studies of TME performed at the University of Wisconsin, Madison have demonstrated experimental transmission to sheep, goats, striped skunk, squirrel monkey, stump-tailed and rhesus monkey, and hamster as reviewed by Rhein *et al*[47]. TME in hamsters presents as two different clinical pictures with unique incubation periods, histological lesions, and biochemical profiles. The two strains are referred to as 'hyper' and 'drowsy', and reflect the manifestations of clinical disease[48].

Clinical signs

The incubation period of natural TME has been estimated at 7–12 months, based on observations following epizootics. Initially, infected mink display behavioural changes including increased aggressiveness and hyperesthesia which progresses to ataxia, occasionally tremors or circling, and compulsive biting of self or objects (Fig. 3)[47]. Clinical signs usually progress over weeks but can range from 1 week to several months prior to death[42].

Histopathology

The most salient histological feature in the TME brain is the extensive neuropil vacuolation. Additionally, there is neuronal degeneration and astrocytosis characteristic of TSEs. Lesions are well developed in the cerebral cortex, particularly in the frontal cortex, as well as the corpus

striatum, thalamus and hypothalamus, and are less severe in the midbrain, pons and medulla. Spongiform change is not usually evident in the cerebellum and spinal cord[47].

In contrast to CWD, little evidence of prion infection can be detected in extraneural tissues of TME-infected mink. However, low concentrations of infectivity have been demonstrated in spleen, intestine, and mesenteric lymph node by bioassay[49].

Prevention of TME

Similar to FSE, TME apparently has arisen from exposure to a food-borne prion agent, likely scrapie or a BSE-like agent in downer cattle. The heightened awareness of TME by mink ranchers has likely led to the exclusion of sheep or cattle as a food source. Therefore, future TME infections are expected to be exceedingly rare.

Acknowledgements

We thank our colleagues for their contributions and helpful discussions: D Gould, C Mathiason, J Bartz, J Fischer, R Allison, M Perrott, E Hoover, T Spraker, E Williams, L Creekmore, and Katherine O'Rourke. This work was supported, in part, by a grant from the National Institutes of Health.

References

1 Kirkwood JK, Cunningham AA. Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles. *Vet Rec* 1994; **135**: 296–303
2 Pearson GR *et al*. Feline spongiform encephalopathy: fibril and PrP studies. *Vet Rec* 1992; **131**: 307–10
3 Taylor DM, Woodgate SL. Bovine spongiform encephalopathy: the causal role of ruminant-derived protein in cattle diets. *Rev Sci Tech* 1997; **16**: 187–98
4 Bons N *et al*. Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. *Proc Natl Acad Sci USA* 1999; **96**: 4046–51
5 Williams ES, Kirkwood JK, Miller MW. In: Williams ES, Barker IK. (eds) *Infectious Diseases of Wild Mammals*. Ames, Iowa: Iowa State University Press, 2001; 292–312
6 Kirkwood JK, Cunningham AA, Wells GA, Wilesmith JW, Barnett JE. Spongiform encephalopathy in a herd of greater kudu (*Tragelaphus strepsiceros*): epidemiological observations. *Vet Rec* 1993; **133**: 360–4
7 Bruce M *et al*. Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philos Trans R Soc Lond B Biol Sci* 1994; **343**: 405–11
8 Fraser H *et al*. Transmission of feline spongiform encephalopathy to mice. *Vet Rec* 1994; **134**: 449
9 Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD [see comments]. *Nature* 1996; **383**: 685–90
10 Ryder SJ, Wells GA, Bradshaw JM, Pearson GR. Inconsistent detection of PrP in extraneural tissues of cats with feline spongiform encephalopathy. *Vet Rec* 2001; **148**: 437–41

11 Wyatt JM *et al*. Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats. *Vet Rec* 1991; **129**: 233–6
12 Bratberg B, Ueland K, Wells GA. Feline spongiform encephalopathy in a cat in Norway. *Vet Rec* 1995; **136**: 444
13 Nathanson N, Wilesmith J, Wells GA, Griot C. In: Prusiner SB. (ed) *Prion Biology and Diseases*. New York: Cold Spring Harbor Laboratory Press, 1999; 431–63
14 Williams ES, Young S. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J Wildl Dis* 1980; **16**: 89–98
15 Williams ES, Young S. Spongiform encephalopathy of Rocky Mountain elk. *J Wildl Dis* 1982; **18**: 465–71
16 Spraker TR *et al*. Spongiform encephalopathy in free-ranging mule deer *(Odocoileus hemionus)*, white-tailed deer *(Odocoileus virginianus)* and Rocky Mountain elk *(Cervus elaphus nelsoni)* in northcentral Colorado. *J Wildl Dis* 1997; **33**: 1–6
17 Miller MW *et al*. Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. *J Wildl Dis* 2000; **36**: 676–90
18 Williams ES, Miller MW. Chronic wasting disease in deer and elk in North America. *Rev Sci Tech* 2002; **21**: 305–16
19 Williams ES, Young S. Spongiform encephalopathies in Cervidae. *Rev Sci Tech* 1992; **11**: 551–67
20 Miller MW, Wild MA, Williams ES. Epidemiology of chronic wasting disease in captive Rocky Mountain elk. *J Wildl Dis* 1998; **34**: 532–8
21 Spraker TR *et al*. Comparison of histological lesions and immunohistochemical staining of proteinase-resistant prion protein in a naturally occurring spongiform encephalopathy of free-ranging mule deer (*Odocoileus hemionus*) with those of chronic wasting disease of captive deer. *Vet Pathol* 2002; **39**: 110–9
22 Miller MW, Williams ES. Detecting PrP^{CWD} in mule deer by immunohistochemistry of lymphoid tissues. *Vet Rec* 2002; **151**: In press
23 Gross JE, Miller MW. Chronic wasting disease in mule deer: disease dynamics and control. *J Wildl Manag* 2001; **65**: 205–15
24 Fischer J, Nettles V, Creekmore L. In: St Louis, MO: Proceedings of the 106th Meeting of the United States Animal Health Association, 2002
25 Williams ES, Young S. Neuropathology of chronic wasting disease of mule deer (Odocoileus hemionus) and elk (Cervus elaphus nelsoni). Vet Pathol 1993; **30**: 36–45
26 Eckroade RJ, ZuRhein GM, Hanson RP. In: Prusiner SB, Hadlow WJ. (eds) Slow Transmissible Diseases of the Nervous System. New York: Academic Press, 1979; 409–41
27 Sigurdson CJ et al. Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns (Odocoileus hemionus). J Gen Virol 1999; **80**: 2757–64
28 Sigurdson CJ et al. PrP^{CWD} lymphoid cell targets in early and advanced chronic wasting disease of mule deer. J Gen Virol 2002; **83**: 2617–28
29 Sigurdson CJ, Spraker TR, Miller MW, Oesch B, Hoover EA. PrP^{CWD} in the mysenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease. J Gen Virol 2001; **82**: 2327–34
30 Wild MA, Spraker TR, Sigurdson CJ, O'Rourke KI, Miller MW. Preclinical diagnosis of chronic wasting disease in captive mule deer (Odocoileus hemionus) and white-tailed deer (Odocoileus virginianus) using tonsillar biopsy. J Gen Virol 2002; **83**: 2629–34
31 Wolfe LL et al. Evaluation of antemortem sampling to estimate chronic wasting disease prevalence in free-ranging mule deer. J Wildl Manage 2002; **66**: 564–73
32 Kimberlin RH, Walker CA. Pathogenesis of scrapie in mice after intragastric infection. Virus Res 1989; **12**: 213–20
33 Bruce M, Chree A, Williams ES, Fraser H. In: Birmingham, UK: XIVth International Congress of Neuropathology, 2000
34 Bartz JC, Marsh RF, McKenzie DI, Aiken JM. The host range of chronic wasting disease is altered on passage in ferrets. Virology 1998; **251**: 297–301
35 Hamir AN, Miller JM. In: Denver, CO: Chronic Wasting Disease Symposium 14, 2002
36 Belay ED et al. Creutzfeldt-Jakob disease in unusually young patients who consumed venison. Arch Neurol 2001; **58**: 1673–8

37 Raymond GJ et al. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. EMBO J 2000; **19**: 4425–30
38 Hamir AN et al. Preliminary findings on the experimental transmission of chronic wasting disease agent of mule deer to cattle. J Vet Diagn Invest 2001; **13**: 91–6
39 Gould DH et al. Survey of cattle in northeast Colorado for evidence of chronic wasting disease: geographical and high risk targeted sample. J Vet Diagn Invest 2002; Submitted
40 O'Rourke KI et al. PrP genotypes of captive and free-ranging Rocky Mountain elk (Cervus elaphus nelsoni) with chronic wasting disease. J Gen Virol 1999; **80**: 2765–9
41 Will RG et al. Diagnosis of new variant Creutzfeldt-Jakob disease. Ann Neurol 2000; **47**: 575–82
42 Marsh RF, Hadlow WJ. Transmissible mink encephalopathy. Rev Sci Tech 1992; **11**: 539–50
43 Marsh RF, Bessen RA. Epidemiologic and experimental studies on transmissible mink encephalopathy. Dev Biol Stand 1993; **80**: 111–8
44 Hanson RP et al. Susceptibility of mink to sheep scrapie. Science 1971; **172**: 859–61
45 Marsh RF, Hanson RP. In: Prusiner SB, Hadlow WJ. (eds) Slow Transmissible Diseases of the Nervous System. New York: Academic Press, 1979; 451–60
46 Marsh RF, Bessen RA, Lehmann S, Hartsough GR. Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. J Gen Virol 1991; **72**: 589–94
47 Rhein GMZ, Eckroade RJ, Grabow JD. In: Zeman W, Lennett E, Brunson J. (eds) Slow Virus Diseases. Baltimore, MD: Williams and Wilkins, 1974; 16–38
48 Bessen RA, Marsh RF. Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol 1992; **73**: 329–34
49 Hadlow WJ, Race RE, Kennedy RC. Temporal distribution of transmissible mink encephalopathy virus in mink inoculated subcutaneously. J Virol 1987; **61**: 3235–40