

CHRONIC WASTING DISEASE REVIEW

Written by Dr Debra Bourne, Wildlife Information Network, October 2004, for SEAC

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RESOURCES USED IN WRITING THIS REVIEW

This review was based on a peer-reviewed Wildlife Information Network CD-ROM (Wildpro v5.0 Chronic Wasting Disease in Deer and Elk (Bourne, D.C., Dein, F.J. & Boardman, S.I. (eds.). Wildlife Information Network, Twycross, UK. ISBN 0-9547185-4-

2. This volume of Wildpro is accessible at www.wildlifeinformation.org and was based on references found by the following methods:

- Searches on PubMed using various keywords including “CWD”, “Chronic Wasting Disease” and “transmissible spongiform encephalopathy”, together with other keywords (transmissible mink encephalopathy/TME, feline spongiform encephalopathy/FSE etc.) for comparative data. Searches were also carried out using names of key authors, e.g. “O’Rourke” and “Williams, ES”.
- Searches on Agricola, Biological Abstracts, CAB Abstracts, Wildlife Worldwide and Zoological Record. Keywords used included chronic wasting disease, CWD, transmissible spongiform encephalopathies, bovine spongiform encephalopathy, scrapie, transmissible mink encephalopathy, fatal familial insomnia, kuru, Gerstmann-Straussler-Scheinker, feline spongiform encephalopathy and nvCJD.
- Abstracts of papers found from database searches were read and relevant papers (all papers on CWD plus key and review papers on other TSEs/general prion science) were then examined in more detail.
- For key journals such as Emerging Infectious Diseases and Journal of General Virology, the contents list of every volume was checked, or keyword searches were performed on the website of the individual journal.
- Reference lists of papers on CWD were traced backwards, particularly those from both recent and early review articles on the topic. Similar tracing back was performed for papers on other TSEs and general papers on TSEs/prion science, where this was felt appropriate.
- Key proceedings were checked, e.g. proceedings of conferences on CWD/TSEs and certain other conferences such as the Proceedings of the Wildlife Disease Association and the US Animal Health Association.
- Reference lists on CWD/TSEs compiled by other organisations were checked, particularly that of the National Wildlife Health Center, USGS
- ProMED-Mail was utilised as a means of detecting emerging information, for example initial reports of findings of CWD in new geographical areas.
- For data on geographical range and incidence, relevant federal and state/provincial websites were searched, e.g. CFIA, APHIS, Colorado Fish & Game, Wisconsin Department of Natural Resources. Such sites also provided additional material such as the Wisconsin DNR’s “Environmental Impact Statement” and “An Analysis of Risks Associated with the Disposal of Deer from Wisconsin in Municipal Solid Waste Landfill.”
- Published data from journals, books, proceedings and Websites was supplemented by personal communications from people working on CWD in Wisconsin and elsewhere in the USA

In addition, for this report, data specifically concerning CWD was updated by searches on PubMed, tracking back from recent papers, checking key websites and key journals and using some more general web searches (on Google) using carefully chosen keyword combinations. Very recent findings were confirmed or expanded, where required, by contacting key researchers.

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1) INTRODUCTION

1. Chronic wasting disease (CWD) is one of the group of diseases generally known as the transmissible spongiform encephalopathies (TSEs) or prion diseases. Other known TSEs include scrapie of sheep and goats, transmissible mink encephalopathy (TME) of farmed mink, Creutzfeldt-Jakob disease (CJD), kuru, fatal familial insomnia (FFI) and Gerstmann-Sträussler-Scheinker syndrome (GSS) in humans, and bovine spongiform encephalopathy (BSE, colloquially known as “mad cow disease”) in cattle (Phillips, Bridgeman & Ferguson-Smith, 2001). TSE in captive exotic Bovidae in the UK, feline spongiform encephalopathy (FSE) in domestic and exotic cats (Felidae) and variant CJD (vCJD or nv CJD – new variant CJD) in humans are all considered to be derived from BSE (Phillips, Bridgeman & Ferguson-Smith, 2001; Williams, Kirkwood & Miller, 2001). CWD is the only known prion disease of free-living non-domestic animals. CWD is considered important due to (a) the possible effect of the disease on free-living populations of native cervids in North America; (b) the impact of disease control measures on both wild populations and the cervid farming industry in North America; (c) the potential impact of both the disease and control measures on cervid hunting and other wildlife-related recreation in North America; (d) concerns regarding possible implications for domestic animal (e.g. cattle, sheep) health; and (e) concerns regarding possible implications for human health (Bourne, 2004a; Bollinger *et al.*, 2004; Miller & Williams, 2004). There is also concern that CWD could become established in farmed and/or free-living cervids outside North America.

2) EMERGENCE, AETIOLOGY AND POSSIBLE ORIGINS

Emergence/History

2. Chronic wasting disease was first recognised as a clinical syndrome, “chronic wasting disease” in mule deer (*Odocoileus hemionus hemionus*) at a research facility in Fort Collins, Colorado, USA in 1967 (Williams & Young, 1980; Williams & Young, 1992). The disease, initially considered to be related to captivity and associated with nutritional deficiency, toxins or stress, was recognised as a spongiform encephalopathy in 1978 (Williams & Young, 1980; Williams & Young, 1992). CWD was recognised clinically in research facilities in Wyoming, USA, in 1978 (Williams & Young, 1992). In 1979, based on samples taken in 1978, the disease was confirmed in mule deer and the subspecies black-tailed deer *Odocoileus hemionus columbianus* in the research facilities in Wyoming (Williams, Miller & Thorn, 2002). Also in 1979, CWD was diagnosed for the first time in captive Rocky Mountain elk (*Cervus elaphus nelsoni*) in the same facilities in which the disease was known in mule deer (Williams & Young, 1982).

3. The first diagnosis of CWD in wild cervids occurred in Rocky Mountain elk in Colorado in 1981; the first diagnosis in free-ranging mule deer occurred in 1985, and CWD was diagnosed in free-ranging white-tailed deer *Odocoileus virginianus* in 1990 (Williams, Miller & Thorne, 2002). A review of archived records from the Colorado Department of Wildlife has revealed a probable case of clinical CWD (misdiagnosed at that time) in a free-ranging mule deer from 1978 (Miller *et al.*, 2000).

4. Within farmed cervids, the disease was first diagnosed in a game farm elk in Saskatchewan, Canada, in 1996 and in game farm elk in South Dakota, USA, in 1997 (ProMED-Mail, 1996; Williams, Miller & Thorne, 2002; Williams *et al.*, 2002).

5. It is still not known, and it may never be possible to determine retrospectively, whether CWD originated within captive cervids in research facilities and spread to free-ranging cervids, or was introduced into the facilities from the wild, or indeed, whether it arose separately in captive and free-ranging cervids (Spraker *et al.*, 2002c; Williams & Miller, 2003).

Aetiology

6. CWD has been recognised as a spongiform encephalopathy since 1978, based initially on the characteristic neuropathological lesions (including widespread spongiform change of the neuropil, intracytoplasmic vacuoles in neuronal perikaryons, and hypertrophy and hyperplasia of astrocytes) of individuals with the clinical syndrome “chronic wasting disease” (Williams & Young, 1980, Williams & Young, 1982) and later also on the presence of scrapie-associated fibrils in the brain and/or spleen of affected deer and elk (Williams & Young, 1992) and the presence of PrP^{res} in the brain (Guiroy *et al.*, 1991a; 1991b).

7. CWD was first shown to be transmissible by intracerebral inoculation into ferrets (two to three months old at inoculation) and weanling hand-reared mule deer fawns (Williams, Young & Marsh, 1982).

8. Available data indicates that the CWD agent is different from the agents of BSE, TME, CJD and known scrapie strains. Data from strain typing by incubation period and lesion profiles following inoculation into genotypically characterised mice indicate that CWD agent is different from BSE agent, TME agent and from tested strains of CJD agent and scrapie agent (Bruce *et al.*, 1997; Bruce *et al.*, 2000, Laplanche *et al.*, 1999). In mice, using CWD serially passaged in mice (initial transmission to mice is unreliable – see discussion below in paragraph 61), the incubation period following intracerebral inoculation varied according to the PrP genotype of the inoculated mice. Pathologically, vacuolar degeneration of the neurons and neuropil was minimal while the predominant neuropathological finding was extensive perivascular amyloid deposits of PrP; this pathology was targeted to defined brain nuclei. This neuropathological pattern was maintained up to the third mouse-to-mouse passage of CWD and was present in all mouse genotypes tested; it was noted that lesions were most severe in genotypes with a longer incubation period for this TSE. The incubation periods and histopathological findings were noted to be “markedly different” from any seen previously in mice inoculated with isolates derived from BSE, CJD or scrapie, indicating that the strain of agent involved was different from those tested previously (Bruce *et al.*, 2000).

9. When glycoform patterns of PrP-res from CWD infected mule deer, white-tailed deer and elk were compared with one another and with PrP-res from scrapie-infected sheep, scrapie-infected cattle and BSE-infected cattle, it was found that the strongest PrP-res signal for all the cervids was in the upper, diglycosylated band at 30-kDa and the weakest signal for all the cervids was in the lowest, unglycosylated band at 22-kDa; some variation was noted between individuals within a species. The authors concluded that the pattern of glycosylation was not sufficiently distinct to allow reliable differentiation between CWD, scrapie and BSE (Race *et al.*, 2002).

Strains of CWD?

10. The question of whether there are one or several strains of CWD has not yet been answered. Both the marked similarity of the lesions, and epidemiological data, strongly suggest that the same CWD agent is responsible for the disease in captive and free-ranging deer and elk (Williams & Miller, 2002).

11. In the study by Race *et al.*, analysis of the proportions of the 30-kDa versus 22-kDa bands on a scatter graph resulted in tighter grouping for the elk than for the deer and a suggestion was made that perhaps the elk were infected with a single strain of the disease while different strains infected the mule deer. Alternatively the variation might simply reflect the fact that the population of animals is not homogenous (Race *et al.*, 2002). In another study, Western blot profiles from codon 132 M/M and L/L homozygous (in the PRNP gene)

elk were found to be indistinguishable, with the characteristic three bands detected, but in two L/M heterozygotes a distinctive pattern was detected with the two upper bands having a higher apparent molecular weight than the upper two bands from an M/M homozygote, while the lowest band detectable only after the blot was exposed for a long time. In the discussion it was noted that, due to variation in the precise anatomical location of tissue used in the Western blot analysis, and the small sample size, a conclusion that the difference in glycoform ratio was due to a difference in genotype would be premature (Spraker *et al.*, 2004).

12. Data from transmission studies into laboratory animals are inconclusive. In recent ferret studies it was noted that when different sources of CWD material were used, the patterns of transmission route susceptibility, incubation time and distribution of PrP^{CWD} differed, supporting the possibility of the existence of different strains of CWD (Perrott *et al.*, 2004). However, studies in transgenic mice expressing cervid PrP indicated that the same prion strain was responsible for infection in analysed elk and mule deer (Browning *et al.*, 2004).

13. Now that models for bioassay have been developed (ferrets and transgenic mice) (Browning *et al.*, 2004; Kong *et al.*, 2004; Perrott *et al.*, 2004); further information on the presence or otherwise of CWD strains, either within or between natural host species, may become available.

Possible Origins of CWD

14. The exact origin of CWD is not known and cannot be definitively determined using techniques which are currently available (Williams & Miller, 2003). There are three main theories regarding the possible origins of CWD: that it arose from a spontaneous somatic mutation or spontaneous conformational alteration of the prion protein in mule deer, that it originated from scrapie, or that it originated from an unrecognised prion strain from an unknown source (Williams & Miller, 2002; Williams & Miller, 2003; Williams, Miller & Thorne, 2002; Salman, 2003). It is considered most probable, based on data on prevalence and distribution, and using mathematical modelling, that CWD may have arisen in northeastern Colorado or southeastern Wyoming and may have been present in parts of these areas from the early 1960s, or even earlier (Miller *et al.*, 2000).

Scrapie

15. Scrapie has been a known disease of sheep in the USA for many years. It is possible that due to some unknown factor the causative agent of scrapie became able to infect mule deer and became adapted to cervids (Williams & Miller, 2002; Williams *et al.*, 2002; Race *et al.*, 2002). Passage through another species has been documented to change the host range of TSEs (e.g. Marsh & Hadlow, 1992; Bartz *et al.*, 1998).

16. Domestic goats and sheep were occasionally housed at the research facilities in which CWD was first described. Reports of scrapie in domestic sheep in Colorado or Wyoming prior to the late 1980s were rare, with only one report from Colorado and two reports from Wyoming during 1947-1977; these cases occurred in counties which were far from the research facilities and the area now known as the CWD endemic area (Hourrigan *et al.*, 1979). It is probable that scrapie was under recognised and underreported in the USA at this time (Williams & Young, 1992). Free-ranging cervids share high mountain grazing areas with sheep during the summer and sometimes feed from hay racks provided for domestic stock during the winter (MacDiarmid, 1990).

17. In support of this theory, it should be noted that scrapie transmitted to elk by intracerebral inoculation produced central nervous system (CNS) lesions which were not distinguishable from CWD by histopathology or immunohistochemistry. Scrapie was inoculated intracerebrally into six Rocky Mountain elk *Cervus elaphus nelsoni*; a further two elk acted as uninoculated controls. Three animals which died or were euthanased for reasons unconnected to the study during the first two years post inoculation did not show any histopathological lesions indicative of a TSE and neither scrapie associated fibrils nor proteinase-K resistant PrP was detected (Hamir *et al.*, 2003b, Hamir *et al.*, 2004b). In the third and fourth years post inoculation, the other three inoculated elk died (at 25, 35 and 46 months post inoculation) after brief episodes of neurological signs (falling and paddling of the legs while in lateral recumbency). Slight behavioural changes had occurred in the first of these animals and slight reduction of appetite leading to some weight loss in the later two animals (Hamir *et al.*, 2004b). At necropsy, the only gross lesion was moderate weight loss. Microscopically, vacuolation of neuronal perikarya and neuropil were present in the brain and spinal cord, together with mild multifocal increases in glial cells, but without prominent neuronal degeneration. Brain lesions were most severe in the thalamus and cerebellum. In the brainstem, while spongiform lesions were seen in the dorsal motor nucleus of the vagus more severe lesions were present in the pontine nucleus. By immunohistochemistry, PrP was detected in cerebrum, cerebellum, brainstem, multiple spinal cord regions, in diffuse neurons in the Gasserian (trigeminal) ganglia (one of three) and in the retinas, but not the optic discs or optic nerves, of all three elk. IHC showed staining in the cerebrum, cerebellum, brainstem and spinal cord. Staining was diffusely distributed throughout the grey matter neuropil in all parts of the CNS, including, in the cerebellum, both the granular and molecular layers. A primarily punctate and granular staining pattern was noted with some small aggregates but few accumulations sufficiently large to be described as plaques. In neurons generally PrP staining was perineuronal, forming a ring around the surface of the cell, but large stained granules were visible in the perikaryon of some neurons (Hamir *et al.*, 2003b; Hamir *et al.*, 2004b). In general, the pattern of lesions was considered similar to that seen in elk with CWD. CNS tissues were positive for proteinase-K resistant PrP by immunohistochemistry and by Western blotting (Hamir *et al.*, 2004b).

18. Additionally in cell-free conversion experiments PrP^{CWD} converted ovine PrP with an efficiency intermediate between its conversion of human or bovine PrP and its conversion of cervid PrP (Raymond *et al.*, 2000).

19. However, it should be noted also that data from strain typing by incubation period and lesion profiles following inoculation into genotypically characterised mice indicate that CWD agent is different from tested strains of scrapie agent (Bruce *et al.*, 1997; Laplanche *et al.*, 1999; Bruce *et al.*, 2000).

Development from a spontaneous or genetic SE in mule deer

20. It is possible that CWD arose from a spontaneous somatic mutation of the PRNP gene in mule deer, followed by transmission to other mule deer and to elk and white-tailed deer (Williams & Miller, 2002; Salman, 2003) or by a spontaneous conformational alteration of PrP^C to PrP^{res}, followed by transmission (Williams, Miller & Thorne, 2002; Williams *et al.*, 2002; Salman, 2003). If CWD arose *de novo* from a new mutation in cervids, then it is most likely that it occurred first in mule deer: in the wildlife research facilities in Colorado and Wyoming in which the disease was first noted, detection of CWD in elk followed detection in mule deer by about two years thus, if the incubation period in the two species is taken to be similar, then the mule deer transmitted the disease to the elk (Williams & Young, 1992). If CWD did arise from a spontaneous TSE in deer then this may have occurred either in the wild or in captivity (Williams, Miller & Thorne, 2002).

Unknown source

21. The third possibility is that CWD originated from infection of cervids with a, as yet unrecognised, prion strain from an unknown source (Williams & Miller, 2002; Williams *et al.*, 2002; Salman, 2003). There is no data to either support or reject this hypothesis.

Alternative suggestions considered and rejected

Familial or genetic association

22. Deer involved in the early outbreaks in the research facilities in Colorado and Wyoming originated from various sources: deer hand-reared on-site having been captured as healthy neonates, deer captured as apparent orphans and hand-reared elsewhere for days to months before being handed to the research facilities, animals captured as adults from throughout Wyoming and Colorado (plus the black-tailed deer *Odocoileus hemionus columbianus* from Oregon), deer born to does resident within the facilities and dam-reared within the facilities, deer born to does within the facilities and taken for hand-rearing at about two days old, and deer born to wild-caught does which were kept only until they had given birth, then released when the fawns were about two days old, the fawns then being hand-reared (Williams & Young, 1992).

23. Origins of affected elk at the Fort Collins and Wyoming facilities included animals born in the wild and either caught as healthy neonates or rescued as presumed orphans, then hand-reared at the facilities, animals born in the wild and captured as adults, animals born to dams resident in the facilities, and dam-raised, and animals born in a zoological park and hand-raised at the Fort Collins facility (Williams & Young, 1992).

24. Most of the affected animals in these early outbreaks were not related to one another. Due to the diverse geographic origins of the cervids it was considered that there was no evidence of any familial or genetic association (Williams & Young, 1992).

Animal protein fed to the deer

25. Available management data indicate that, except for milk (fresh, canned evaporated, buttermilk and occasionally commercial lamb milk replacer) used in hand-rearing fawns, no animal protein was fed to any of the deer kept in the Colorado or Wyoming research facilities. Diets included grass, hay, other vegetation, dried high protein alfalfa hay and grain mixtures, as well as multivitamins, salt and mineral blocks. Sources of hay and of grain mixtures used were different for the Colorado and Wyoming facilities. Overall, it was considered that there was no evidence of CWD being associated with feed; it was acknowledged that detailed data on feeding was not available for the period before 1974, but it was considered probable that management was similar (Williams & Young, 1992).

26. An epidemiological study of the disease in a cohort of elk at a research facility (Foothills Wildlife Research Facility, Fort Collins, Colorado) eliminated animal protein as a source of infection since no animal protein was present in diets fed to the elk (Miller, Wild & Williams, 1998).

3) KNOWN GEOGRAPHICAL DISTRIBUTION AND TIMELINES OF SPREAD

In captive cervids

27. Within captive cervids at least three different populations must be considered: cervids in research facilities, cervids in zoos, and farmed cervids.

In research facilities

28. As indicated above (paragraph 2), CWD was first identified in *Odocoileus hemionus* – mule deer at a research facility near Fort Collins, Colorado, in 1967 and recognised as a TSE in 1978; cases in mule deer at a research facility in Wyoming were first confirmed in 1979 and cases in elk were confirmed in the research facilities in the same year. Deer in the research facility in Fort Collins, Colorado, in which CWD was first identified originated from free-ranging populations as well as from the wildlife research facility in Wyoming at which the disease was also seen; only a few deer were transferred in the opposite direction. A second Colorado facility first recorded the disease about 18 months after a deer had been accepted from a facility with CWD (Williams & Young, 1992; Williams & Miller, 2002).

29. For the two main research facilities in Colorado and Wyoming in which the disease was originally recognised, it is not possible to say whether the disease was transmitted from one facility to the other, and if so, in which direction. Spread to a small facility in Colorado was probably by importation of an animal from a larger facility in which the disease occurred: CWD was first noted in the small facility 18 months after importation of a deer from the CWD-affected facility; this animal then developed CWD (Williams & Young, 1992).

In zoos

30. Few cases of CWD have occurred in zoos. A case was confirmed in a single Rocky Mountain elk *Cervus elaphus nelsoni* at a small zoo in Wyoming; the animal had been hand-reared at a research facility in Wyoming where CWD occurred. CWD was confirmed histologically in mule deer in a zoo in Ontario, Canada; these animals had originated from a zoo in Colorado which had received deer from the Colorado research facility where CWD occurred. CWD was never officially diagnosed at the zoo in Colorado but deer there had developed clinical signs suggestive of the disease (Williams & Young, 1992; Bollinger *et al.*, 2004). CWD does not appear to have become established at these locations (Laplace *et al.*, 1999; Bollinger *et al.*, 2004).

31. The American Association of Zoo Veterinarians has issued guidelines for zoos regarding CWD, including recommendations that the brain from any cervid dying or euthanased at 12 months old or older should be submitted for testing for CWD, as well as guidance to minimise the risk of introduction of CWD into zoological collections (AAZV Infectious Diseases Committee, 2003). There is no recent published data indicating that CWD has been detected in any zoo cervids.

In farmed cervids

Canada

32. Within farmed cervids the disease was first diagnosed in a game farm elk in Saskatchewan, Canada, in 1996 (ProMED-Mail, 1996; Williams, Miller & Thorne, 2002; Williams *et al.*, 2002; Kahn *et al.*, 2004). A second case in farmed elk was diagnosed in 1998 (Kahn *et al.*, 2004). The first detected case in farmed white-tailed deer in Canada was found on a farm in Alberta in November 2002 (Kahn *et al.*, 2004). Further testing revealed the presence of the disease on 40 game farms in Saskatchewan (all elk) and three in Alberta (elk and white-tailed deer) (Kahn *et al.*, 2004; Bollinger *et al.*, 2004). An eradication programme for CWD in farmed cervids was implemented by the Canadian Food Inspection Agency in 2000 (Bollinger *et al.*, 2004). Not all the outbreaks of CWD in farmed cervids in Canada can be fully explained by epidemiological findings. There was apparently an importation of infected elk into a game farm in Saskatchewan in the 1980s or perhaps even earlier and it is possible that one or more additional, undocumented, importations of infected animals or other sources of infection may also have occurred (Bollinger *et al.*, 2004). Due to the structure of the farmed cervid industry in Canada there is general separation of Saskatchewan and Alberta

farmed elk from farmed cervids in other provinces, however further surveillance will be carried out to confirm the absence or presence of CWD elsewhere in Canada (Kahn *et al.*, 2004).

USA

33. CWD has been detected in farmed cervids in Colorado, Kansas, Minnesota, Montana, Nebraska, Oklahoma, South Dakota and Wisconsin (Miller & Williams, 2004).

34. The first detection of CWD in farmed animals in the USA occurred on an elk farm in South Dakota in 1997 (Creekmore, 2002; Holland, 2002). Following investigations, seven herds were quarantined and then depopulated. CWD was then detected in August 2002 in a three-year-old bull elk in a privately owned elk herd in the Black Hills. The ranch was adjacent to a ranch on which the disease had been detected and eliminated 51 months earlier. The CWD-positive animal was born after the other facility was depopulated (Holland, 2002; ProMED-Mail, 2002b; South Dakota Game, Fish and Parks, 2002).

35. In Montana, CWD was detected on a game farm in Philipsburg in 1999; the whole elk herd was depopulated and nine of the 81 animals tested positive for CWD (Montana Department of Livestock, 2003).

36. In Nebraska, CWD has been diagnosed on three facilities. The first (in Cherry county) had one CWD-positive bull elk detected in 1998 (Nebraska Game and Parks Commission, 1998); this herd was declared CWD free in 2001 following three years of surveillance. The second facility, in Cheyenne County, had four CWD-positive elk and was depopulated in 2001. In the third facility, in northern Sioux County, four elk were found CWD-positive during December 2001 to March 2002 with a further seven positives detected when the remaining 74 elk were removed during depopulation. Additionally in a pen adjacent to the elk pen, in which free-living *Odocoileus virginianus* - White-tailed deer had been enclosed when the pen was constructed, 11/21 hunter-harvested white-tailed deer were positive, with 87 of further positive out of 170 individuals when the pen was depopulated (Morrison, 2002).

37. In Oklahoma, two cases diagnosed were diagnosed in farmed elk in 1998 and 1999; both originated from a game farm in Philipsburg, Montana (ProMED-mail, 1999). In 2001 five cases were diagnosed in an elk herd; the elk originally came from Montana (ProMED-mail, 2001a).

38. In Colorado, CWD was detected in elk on three game farms in 2001, two in northern Colorado, within the area where the disease has been endemic in wild cervids for many years and one in south-central Colorado (ProMED-Mail, 2001). The disease was subsequently confirmed in several more game farms or hunting ranches and a number of ranches in the endemic area were depopulated (Ver Steeg, 2003; Miller & Williams, 2004). The latest infected hunting ranch was reported in January 2004; this was the second affected ranch detected in the Western Slope area of Colorado (Colorado Department of Agriculture, 2004).

39. In Kansas, CWD was diagnosed in November 2001 in an elk in a private elk herd near Anthony, Kansas. This animal had been imported from a ranch in Colorado on which CWD was subsequently detected (ProMED-Mail 2002a).

40. In Minnesota, CWD was reported in one captive bull elk which had died “mysteriously” in 2002; depopulation and testing was carried out and the other 48 elk in the herd were all found to be CWD-negative (DelGiudice, 2002).

41. In Wisconsin, CWD was first detected by routine surveillance in a white-tailed deer buck shot on a game farm in Portage County in 2002. During investigations, CWD was then detected in a captive female white-tailed deer from a farm in Walworth County and in a buck, shot near the farm in October 2002, thought to have escaped from the farm in March 2002; four additional infected animals were detected during depopulation of the farm (Bartelt, Pardee & Thiede, 2003). CWD has since been detected on four other white-tailed deer farms and an elk farm (Langenberg, 2004).

Outside North America

42. CWD has been reported from farmed elk in Korea. Elk were shipped from Canada to Korea in 1994 and 1997. These included some animals from a farm which was later discovered to have been infected at the time the elk were exported. One of the exported elk was identified as being CWD-positive in 2001 and the Korean veterinary authorities destroyed all the cervids exposed to the infected elk (B. Peart, pers. comm., 2004). No further cases of CWD have occurred in Korea: all cervids, products and by-products from the farm on which the case occurred in 2001 were destroyed and tested for CWD, with negative results (Sohn *et al.*, 2002).

43. Note that CWD is **not** related to a chronic ill thrift syndrome reported from farmed elk in New Zealand (MacDiarmid, 1990).

In free-ranging cervids

44. Despite extensive surveillance in some states in recent years, relatively little is known about the geographical spread of CWD in free-living cervids, the rate of increase of prevalence in the populations, or the factors affecting these (Bollinger *et al.*, 2004). Surveillance data indicates that CWD occurrence and prevalence varies between states and between regions within a state, and that within affected regions there are clusters of affected animals, i.e. the disease is not evenly spaced (Samuel *et al.*, 2003). It should be noted that surveillance efforts in some geographical areas are not sufficient to detect CWD if it is present at a low prevalence (Bollinger, 2004). It has been suggested that a prevalence of greater than 1% may occur before clinical cases would be detected in the field (Miller *et al.*, 2000) and it has been pointed out surveillance to detect CWD at the 1% infection level means 10,000 infected deer in a population of 1,000,000 (Diefenbach, Rosenberry & Boyd, 2004). Prevalence estimates may be affected by different surveillance strategies (Conner, McCarty & Miller, 2000; Samuel *et al.*, 2003). Targeted surveillance (testing cervids with clinical signs compatible with CWD) may aid in early detection of CWD but does not provide information on prevalence or temporal trends in prevalence (Miller *et al.*, 2000; Samuel *et al.*, 2003; Diefenbach, Rosenberry & Boyd, 2004). It should be noted that even with an effective surveillance programme in place, failure to detect CWD can give a high degree of confidence that CWD is not present above a selected prevalence, but cannot guarantee that the disease is absent from the population (Samuel *et al.*, 2003).

USA

45. By July 2004, CWD had been detected in free-ranging cervids in the USA in eight states, including 19 counties of Colorado, four counties of Illinois, nine counties of Nebraska, one county of New Mexico, nine counties of Nebraska, four counties of South Dakota, three counties of Utah, nine counties of Wisconsin and eight counties of Wyoming (Bollinger, 2004; Cornicelli, 2004). Additionally, publicly-owned deer infected with CWD have been culled from within the confines of elk farms in Colorado, Nebraska and South Dakota (Williams *et al.*, 2002).

46. CWD was first diagnosed in free-ranging cervids in Colorado in 1981 and in Wyoming in 1985. By 1999, 119 cases had been diagnosed in free-ranging cervids from 16 Management Units (MUs) in these states, out of 231 suspected cases (poor body condition and behavioural changes, with or without other indicative signs) submitted in northeastern Colorado and southeastern Wyoming; 109 of these diagnoses were made between 1991 and 1999. Positive animals included 84 of 148 suspect mule deer, seven of 20 suspect white-tailed deer and 28 of 63 suspect elk. No “suspect” cervids (i.e., cervids with clinical signs consistent with CWD) from MUs outside the main affected area were positive (Miller *et al.*, 2000). The estimated prevalence from harvest surveys in the endemic areas of Colorado and Wyoming was less than 1.1% in elk (n=337, harvested 1992-1996) and about 2.5% in deer with an annual range of 0-5.9% (n=687, harvested 1983-1996) (Laplanche *et al.*, 1999). In 2000, it was estimated that prevalence in core management units of the endemic area of Colorado and Wyoming was 6-8% for mule deer and less than 1% in elk, with a prevalence in surrounding management units of less than 1% for both species (Williams, 2000).

47. In northcentral Colorado, between March 1981 and June 1995, 49 cases of CWD were diagnosed (41 mule deer, six rocky Mountain elk and two white-tailed deer). The rate of reporting of cases had increased, possibly mainly due to increased efforts to locate affected animals but also possibly reflecting an increasing incidence and spread of the disease (Spraker *et al.*, 1997). During 2001, within the 19 northeastern game management units (GMUs), estimated prevalence in mule deer ranged from <1% to 11%, average about 5% overall; in elk the estimated prevalence in the same area averaged <1%. It was commented that “*observed trends suggest both prevalence and distribution of CWD in mule deer had slowly increased over the last decade in northeastern Colorado.*” (Miller, 2002). In Routt County in early April 2002, three of 336 wild deer collected within a five-mile radius of an infected elk farm were found positive for CWD. A further three out of 285 deer but none of 135 elk were positive when killed starting 15th April 2002; these results indicated a prevalence of less than 1% in the deer in the area. These were the first CWD-positive wild cervids from west of the continental divide in Colorado (Davidson, 2002). From 30 August 2003 to March 25 2004, 248 CWD-positive cervids were detected in Colorado. This included 204 of 7,260 mule deer (172 inside the CWD “established area” in northeastern Colorado, 32 outside this area), 11 of 343 white-tail deer (nine within the “established area” and two outside) and 33 of 8,064 8,724 elk (16 within and 17 outside the “established area”); 105 moose (*Alces alces*) were all negative. Some CWD-positive animals were detected in areas where none had been detected previously (Colorado Division of Wildlife, 2004a). Estimated prevalence in cervids harvested during the 2003-2004 hunting season varied from 0.6 to 1.3 percent in elk in the northeastern endemic area and 1.2% to nearly 7% in mule deer in the same area, some localised subpopulations having a prevalence of nearly twice the background rates. In northeastern Colorado, estimates were 0.1 to 1.4% for elk and 1% or lower for deer. It was noted that in some areas the number of animals samples was quite low and the confidence intervals around the estimates for these areas were very large (Colorado Division of Wildlife, 2004b).

48. Modelling of the epizootiology of CWD in free-ranging cervids in Colorado and Wyoming suggested that the disease may have been present for more than three decades (Miller *et al.*, 2000).

49. An epizootiological study looked at data on free-ranging cervids in Colorado and Wyoming. It concluded from the pattern of geographic variance in prevalence that the epicentre of highest prevalence was some distance north of Fort Collins, Colorado (where the disease was first detected in captive cervids), and had spread in deer subpopulations “*north and south along the Front and Laramie Range foothills and east via the Laramie/North Platt*

and Cache la Poudre/South Platte river drainages.” It was noted that this was consistent with known corridors for movement and migration of mule deer (Miller *et al.*, 2004).

50. In Wyoming, CWD has been detected in wild cervids since 1985, but was found west of the Continental Divide for the first time in 2002, in two mule deer shot on the western slope of the Medicine Bow Mountains, south of Saratoga and on the western slope of the Sierra Madre northeast of Baggs (ProMED-Mail 2002e). During 2002, CWD was detected in 105 deer and five elk (of 2,550 samples tested), including animals in five hunt areas where it had not previously been documented (Wyoming Game and Fish, 2003a). During 2003, CWD was detected in 156 cervids (of 6,171 sampled), including cervids from seven hunt areas where it had not previously been detected (Wyoming Game and Fish, 2003b). In one Management Unit (MU W64) in Wyoming, CWD was first diagnosed in 1985 and it appears that observed prevalence had risen in the MU from $\leq 2.9\%$ in 1983 to 13% by 1998 (Miller *et al.*, 2000).

51. In South Dakota, testing started in 1997 and the first detected CWD-positive free-living cervid was a female white-tailed deer shot in autumn 2001 (Fowler, 2002). By 20th May 2003, six white-tailed deer, five mule deer and one elk had tested positive (South Dakota Game, Fish and Parks, 2003). By the end of June 2004 the total included 17 deer and five elk; eight of the CWD-positive animals were from Wind Cave National Park (South Dakota Game, Fish and Parks, 2004).

52. In Nebraska, surveillance began in autumn 1997 (Morrison, 2002). CWD was diagnosed for the first time in a mule deer harvested November 2000 in southwestern Kimball County within the Nebraska Panhandle (Nebraska Game and Parks Commission, 2001). To summer 2002, more than 2,900 free-living deer and 150 free-living elk had been tested and a total of 15 animals were found CWD-positive including three mule deer in Kimball County, one mule deer in Cheyenne County, one white-tailed deer in Scotts Bluff County and nine white-tailed and one mule deer in Sioux County (Morrison, 2002). About 50% of wild white-tailed deer which had been enclosed by the fence of an elk farm were found to be CWD-positive; further testing found 7/103 (6.8%) deer positive within a five mile radius of the property, 3.5% of 57 deer positive in the zone 5-10 miles from the property and 1/125 deer positive (an animal found at 11 miles away) in the area 10-20 miles from the property (Davidson, 2002). Two hunter-killed mule deer were the first to test CWD-positive from the 2002 fall hunt in Nebraska; both were from the Nebraska Panhandle; nearly 700 deer were tested from this area. All of 846 deer from eastern Nebraska and 305 deer from central Nebraska were CWD-negative (ProMED-Mail, 2003a). Testing in the 2003-2004 detected 158 CWD-positive animals out of 4,850 sampled, all from six western counties of Nebraska (Nebraska Game and Parks Commission, 2004).

53. In New Mexico, CWD was first detected in spring 2002 in the White Sands Missile Range (in unit 17 of the New Mexico Big Game units) (ProMED-Mail, 2002d). CWD was then diagnosed in three more mule deer from the White Sands Missile Range, and later in two deer from the west slope of the Organ Mountains east of Las Cruces (ProMED-Mail 2003b, 2003c). As of July 2004, seven CWD-positive animals had been detected (New Mexico Department of Game & Fish, 2004).

54. In Utah, the first detected case was in a mule deer buck harvested in Autumn 2002 (Utah Division of Wildlife Resources, 2003a). During 2003, CWD-positive animals were found mainly in the LaSal Mountains east of Moab and Diamond Mountain north of Vernal, but one CWD-positive animal was found in central Utah near the city of Fountain Green. Ten deer tested positive between February and December 2003 (Utah Division of Wildlife

Resources, 2003b). By 10th September 2004, one further mature buck had tested positive (Utah Division of Wildlife Resources, 2004).

55. In Wisconsin, three white-tailed deer bucks, harvested in the 2001 hunting season within three miles of one another in south-central Wisconsin (Iowa and Dane counties, Deer Management Unit 70A) were CWD positive; one had CWD-compatible clinical signs. This was the first detection of CWD east of the Mississippi River. A targeted surveillance program had been in place since 1999, with additional sampling of harvested deer (from areas near elk farm which had received animal from infected farms, areas with high density of elk farms and wild deer, and areas in which reestablishment of wild elk populations was being considered) (Langenberg, Beheler & Walser, 2002; Williams *et al.*, 2002). Testing in March and April 2002 detected a further 15 CWD-positive white-tailed deer (2.9% of the 516 animals tested) in western Dane and eastern Iowa counties; the positive animals were found within a five mile radius of the initial three positive deer (Wisconsin Department of Natural Resources, 2002). As more animals were tested, the area in which CWD-positive animals were detected increased. A three-year-old buck shot in Grant County in southwestern Wisconsin was the first positive deer in Wisconsin outside the 411 square mile area west of Madison in which the disease was already known to exist (ProMED-Mail, 2002c). Out of the first 4,554 deer tested in the 2002 fall hunting season, five CWD-positive deer were detected in the CWD Management Zone buffer area around the known CWD-positive 411 square mile area of Dane, Iowa and Sauk counties. This added Richland County to the list of counties in which CWD had been found. The deer were from locations “geographically close” to the Eradication Zone. All of 2,199 samples from outside the Eradication Zone and Management Zone were CWD-negative (ProMED-Mail, 2003a). In a study of 500 deer of one year old or older in southern Wisconsin in 2002, within an area radius approximately 18 km, from 476 useable samples 15 animals were found to be CWD-positive. Of these, 11 were CWD-positive in the obex and the retropharyngeal lymph node using immunohistochemical staining while four were positive only in the retropharyngeal lymph node. In the north-central region of the sampling area the prevalence was higher than expected: 9.4% of 127 deer (95% CI 5.0% - 16.0%) (Joly *et al.*, 2003). During the 2003-2004 hunting season, 106 CWD-positive deer were detected in the Disease Eradication Zone/Intensive Hunting Zone in southwest Wisconsin (Wisconsin Department of Natural Resources, 2004b). In southern Wisconsin, surveillance has detected 10 CWD-positive deer, in Rock, Walworth and Kenosha counties, north of the border from the area of Illinois in which the disease has been detected (Wisconsin Department of Natural Resources, 2004b). The variable spatial prevalence of CWD is thought to suggest relatively recent introduction of CWD (Langenberg, 2004).

56. In Illinois, CWD was first detected in an ill-looking female white-tailed deer in Winnebago County in 2002 (Illinois Department of Natural Resources, 2002); a total of seven cases were detected during 2002 (Illinois Department of Natural Resources, 2003) and additional surveillance in 2003 and early 2004 brought the total to 64, all in northern Illinois, mainly in Winnebago and Boone counties with a few in McHenry and DeKalb counties. Winnebago, Boone and McHenry counties border southern Wisconsin (Illinois Department of Natural Resources, 2004).

57. Looking at the areas in which CWD has been detected for the first time only recently, it should be noted that while infection of free-living cervids in northwest Nebraska and southwest South Dakota probably originated as spread from captive facilities, the origins of CWD in free-living cervids in other areas has not been identified (Miller & Williams, 2004). Importation of an infected cervid is the most commonly suggested hypothesis for the origin of CWD in free-ranging deer in Wisconsin. There is no direct evidence of a link between CWD in free-living cervids in Wisconsin and CWD in captive cervids but investigations into any

possible link between the disease in the captive and free-living populations in Wisconsin are ongoing (Joly *et al.*, 2003).

Canada

58. In Canada, CWD was detected in free-ranging cervids for the first time in 2000, in a wild *Odocoileus hemionus* in Saskatchewan (Bollinger *et al.*, 2004). Testing of 11,055 cervids in Saskatchewan and 2,892 in Alberta between 1996 and 2002 detected only 12 infected cervids, all in Saskatchewan (Kahn *et al.*, 2004). By the end of the 2003 hunting season this had risen to 34 deer, all in Saskatchewan, but from three fairly discrete locations. Twenty nine of the detected CWD-positive animals have been found in the area of southern Saskatchewan known as Saskatchewan Landing; the other two areas in which CWD-positive cervids have been detected are some distance north of this (Bollinger, 2004). The origin of CWD in these animals is not known; it may be transmission from infected farms by accidental contact between farmed cervids and wild cervids or *de novo* emergence of a sporadic form of CWD in the wild cervids (Kahn *et al.*, 2004). Spill-over from infected farms appears to be the most likely origin (Bollinger *et al.*, 2004).

Outside North America

59. CWD is not known to occur in free-ranging cervids outside North America (Spraker, 2003; Kahn *et al.*, 2004). Only limited surveillance has been carried out for TSEs in free-ranging cervids outside North America. Information on results of surveillance to date is discussed later (paragraphs 207-210).

4) HOST RANGE

Known natural hosts

60. The known natural hosts of chronic wasting disease are *Odocoileus hemionus* (including the subspecies *Odocoileus hemionus hemionus* – mule deer and *Odocoileus hemionus columbianus* – black-tailed deer), *Odocoileus virginianus* (white-tailed deer) and *Cervus elaphus elaphus* (Rocky Mountain elk); disease has also occurred naturally in *Odocoileus hemionus* x *Odocoileus virginianus* hybrids (Williams & Young, 1992; Williams *et al.*, 2002). It is presumed that other subspecies of these species, including the European red deer *Cervus elaphus elaphus*, are also susceptible (Williams, 2001; Williams *et al.*, 2002). [Taxonomic note: some authorities designate the European red deer *Cervus elaphus* as a different species from the North American elk, then designated as *Cervus canadensis*. Some authorities consider that all North American forms of *Cervus elaphus* belong to one subspecies, this subspecies then being designated *Cervus elaphus canadensis*. See Whitehead (1993), Geist (1999) and Groves & Grubb (1987) for taxonomic discussions].

Experimental transmission by intracerebral inoculation

61. Transmission by intracerebral inoculation of a suspension of material from the brain of CWD-affected mule deer has been successful in domestic ferrets (*Mustela putorius fero*), American mink (*Mustela vison*), Squirrel monkey (*Saimiri sciurius*), mule deer (*Odocoileus hemionus hemionus*) and a domestic goat (*Capra hircus*) (Williams, Young & Marsh 1982; Williams & Young, 1992; Williams *et al.*, 1992) and more recently to domestic cattle (*Bos taurus*) and domestic sheep (*Ovis aries*) (Hamir *et al.*, 2003a).

62. Initial intracerebral inoculation of homogenised brain tissue from a CWD affected mule deer into two-to-three-month-old ferrets resulted in development of a progressive neurological disorder by 17 to 21 months post inoculation; animals were euthanased *in extremis* after one to six weeks of clinical signs (Williams, Young & Marsh, 1982). Serial passage in ferrets was noted to reduce the incubation period in this species from 17-21 months to first signs using brain homogenate from a clinically affected mule deer, to eight to nine

months when first passage brain material was used and five months when second or subsequent ferret brain material was used for inoculation (Bartz *et al.*, 1998). In another experiment ferrets developed neurological signs and were euthanased at 14 to 19 months post inoculation; histological lesions of spongiform vacuolation and necrosis of neurons were noted and PrP^{CWD} was detected using both ELISA and Western blot; immunofluorescent staining revealed PrP^{CWD} within neurons and at the surface membranes of astrocytes (Sigurdson *et al.*, 2003).

63. In mule deer, incubation periods were 17 months and 21.5 months (Williams, Young & Marsh, 1982; Williams & Young, 1992). In the domestic goat the incubation period was six years (Williams & Young, 1992).

64. Inoculation into standard laboratory rodents has been only partially successful. When material from a CWD-affected mule deer was inoculated intracranially into laboratory mice (*Mus domesticus*), clinical disease or neuropathological findings of disease developed in only "a very few mice", after incubation periods of more than 500 days. Serial passage in mice produced clinical signs in all mouse strains challenged (Bruce *et al.*, 2000). Intracerebral inoculation of a suspension of material from the brain of a CWD-affected mule deer failed to transmit the disease to golden (Syrian) hamsters (*Mesocricetus auratus*) in several experiments (Williams & Young, 1992; Williams *et al.*, 1992), but it was possible to transmit infection into three of 24 hamsters inoculated by intracranial inoculation of material from ferrets after the second passage of CWD (originally from the brain of a clinically-affected mule deer), into 16 of 20 hamsters using fourth-passage material from ferrets and thereafter into all inoculated hamsters by using brain material suspension derived from hamsters which had developed clinical signs (Bartz *et al.*, 1998). These findings showed that the host range of CWD could be altered by passage through other species (Bartz *et al.*, 1998).

65. Experimental intracerebral inoculation of common raccoon (*Procyon lotor*) kits with CWD has failed to cause detectable infection within five years following inoculation: four kits were inoculated and all four animals were still alive and healthy five years post inoculation; the experiment will continue until six years post inoculation (Hamir *et al.*, 2003c, Hamir, 2004). This contrasts with the results of inoculation with TME agent, which resulted in transmission of infection and clinical illness within six months (Hamir *et al.*, 2004c), or scrapie agent, which resulted in transmission within two years (Hamir *et al.*, 2003c, Hamir *et al.*, 2004c). It has been noted that the differences in incubation periods in raccoon following intracerebral inoculation with different TSEs indicates a possible role for this species in differentiating unknown TSE agents (Hamir *et al.*, 2004c; Hamir, 2004).

66. Experimental transmission to domestic cattle is obviously of great interest. In one experiment, 13 calves were inoculated intracranially with a suspension of brain material from CWD-infected mule deer and three calves were maintained as uninoculated controls. The first two animals in which clinical signs (loss of appetite and weight, behavioural signs) developed showed incubation periods of 22 and 23 months, with clinical course to recumbency of two and three months respectively (Hamir *et al.*, 2001). The experiment was terminated at six years post infection. Infection was demonstrated in five of the 13 inoculated cattle, with PrPres deposition in the CNS, detected by immunohistochemistry and Western blot. The central nervous systems of the other eight inoculated animals and the three control animals remained PrP negative. Microscopic lesions suggestive of a spongiform encephalopathy were subtle in three of the inoculated PrP-positive cattle and absent in the other two PrP-positive animals. It was noted that the experiment demonstrated long times (up to five years) for transmission of CWD to cattle even via intracerebral inoculation and that this suggested that, following oral inoculation with CWD into cattle, amplification of PrPres in CNS tissues may

not occur within the normal lifespan of cattle. Further studies are in progress to determine whether a inoculation of cattle with material from CWD-infected cattle might give a shorter incubation period, greater proportion of affected cattle and different clinical and pathological findings (Hamir *et al.*, 2004a).

67. Experimental transmission into domestic sheep has produced a low rate of transmission. Following intracerebral inoculation into eight lambs, by four years into the experiment two sheep, both QQ at codon 171, had been euthanased; one of the sheep had clinical signs and histopathological lesions of spongiform encephalopathy indistinguishable from those of sheep scrapie and PrP^{Sc} was detected in the brain of that individual. The remaining six sheep (two QQ at codon 171 and two QR at codon 171) remained apparently healthy (Hamir *et al.*, 2003a). At five years post inoculation infection had still been detected in only one sheep (Hamir, 2004).

68. White-tailed deer have been shown to be susceptible to intracerebral inoculation of CWD from elk, white-tailed deer and mule deer, with 60% of inoculated animals becoming CWD-positive by two years post inoculation; the study is ongoing (Hamir, 2004; A.N. Hamir, pers. comm. 2004).

69. Fallow deer (*Dama dama*) have been inoculated intracerebrally with CWD from elk and from white-tailed deer; no infection has resulted after two years. The study is ongoing (Hamir, 2004).

70. A study of intracerebral inoculation of CWD into pigs is to start shortly and transmission studies into reindeer (*Rangifer tarandus*) are planned for 2005 (A. N. Hamir, pers. comm. 2004).

71. Obviously, experimental inoculation of humans cannot be attempted for ethical reasons, however transgenic mice expressing human PrP have recently been tested by intracerebral inoculation of elk CWD material. None of the “humanized” mice developed any signs of neurodegenerative disease by 386 days post inoculation. This was in contrast to transmission into transgenic mice expressing cervid PrP by 118 days (average) post inoculation and transmission of sCJD to the humanized mice after 291 days (average) (Kong *et al.*, 2004).

Experimental transmission by oral inoculation

72. CWD has been transmitted by oral inoculation into mule deer, white-tailed deer and elk, giving incubation periods similar to or shorter than those observed for naturally transmitted disease (Williams, 2001); onset of clinical disease occurred at 12 to 34 months in orally infected elk (Williams & Miller, 2002). In one experiment, mule deer fawns from 100 km outside the CWD-endemic area were inoculated orally (5 ml of a 4% w/v homogenate of brain tissue from naturally-infected mule deer, inoculated using a small syringe into the oral diastema and allowing the fawn to lick and swallow the material) for five days. One fawn was killed at each of 10, 42, 53, 77, 78 and 80 days post inoculation. PrP^{Sc} staining was detected (using monoclonal antibody F89/160.1.5) in lymphoid follicles of lymphoid tissues associated with the alimentary tract (retropharyngeal lymph node and/or tonsil and/or Peyer’s patch and/or ileocaecal lymph node) at all times from 42 days onward. No staining was found in control calves which were inoculated with similar material from CWD-negative mule deer and no staining was detected in any neural tissues (Sigurdson *et al.*, 1999).

73. Oral inoculation of 11 cattle with CWD-infected brain tissue (the same inoculum used for successful transmission studies in cervids (Williams, 2001), using a single dose which

would easily result in CWD if given to cervids, has failed to cause any detectable infection after more than six years; the study is ongoing (Woodbury, 2001; Belay et al., 2004).

74. In an ongoing study, one female moose (*Alces alces*) orally inoculated with CWD-infected brain, which died from unrelated causes 465 days post-inoculation, was found to be PrP positive by immunohistochemical examination of the obex (Bollinger *et al.*, 2004; T. Kreeger, pers. comm., 2004). No further details are available at this time.

75. Ferrets, which have been shown to be susceptible to CWD of deer origin by intracerebral inoculation, were not susceptible to CWD of deer origin by oral inoculation. However following passage in ferrets, infection by both oral and intraperitoneal routes was demonstrated (Perrott *et al.*, 2004). After oral inoculation with ferret-passaged CWD, PrP^{CWD} was detected in Peyer's patches, mesenteric and retropharyngeal lymph nodes, and spleen, as well as being widespread in the CNS, but was not detected in the tonsils (Perrott *et al.*, 2004).

Experimental transmission by contact

76. Twenty four cattle have been observed while living with CWD-infected deer herds; after more than six years, no infection has been detected; the study is ongoing (Belay et al., 2004). Other cattle, sheep and goats living in close proximity to CWD-infected cervids in research facilities have, to date, not developed any prion disease (Belay et al., 2004; Miller & Williams, 2004). In a study initiated in fall (autumn) 1998 in a CWD endemic area of northeast Colorado, adult cattle from 22 ranches on which cattle are known to co-mingle with free-living cervids have been tested for CWD following slaughter for age-related problems or due to failure to get in calf. All tested cattle were at least four years old and had been present in the herd for at least four years (mean age of samples cows 8.2 years, median age 8 years, range 4-16 years; time in the herd mean 7.5 years, median 7 years, range 4-16 years). Brains were examined by histopathological examination and by immunostaining using anti-PrP monoclonal antibody F99/97.6.1; no indications of CWD or any other TSE were detected in 262 cattle tested in this way by 2000 (Gould, 2000, Gould *et al.*, 2003). It was noted that, although the findings indicated that it was unlikely that large-scale spread of CWD has occurred to cattle in this area, with a probability of zero or near-zero of not finding an infected animal if the prevalence in the cattle was 2% or higher, the probability of the sample not having detected any diseased animal was higher if the disease prevalence was 1% or lower. It was also noted that several other assumptions were made in determining prevalence limits from this data, such as lack of clustering of disease within herds (Gould *et al.*, 2003).

Natural transmission by contact/environmental contamination

77. A wide variety of species exposed to CWD-agent naturally in research facilities in which the disease is endemic have not developed CWD. Ruminants which have been exposed in this way but remained CWD-negative include domestic cattle, domestic goats, domestic sheep, mouflon (*Ovis orientalis*), pronghorn antelope (*Antilocapra americana*), a blackbuck (*Antelope cervicapra*), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), American bison (*Bison bison*) and moose (*Alces alces*), although the numbers of individuals of each species naturally exposed at such sites has been relatively small, limiting the conclusions which can be made based on these negative findings. CWD also has not been detected in rodents such as deer mice (*Peromyscus maniculus*) at these facilities (Bartelt, Pardee, & Thiede, 2003; Williams, 2001; Miller & Williams, 2004).

5) CWD IN THE KNOWN NATURAL HOSTS

Clinical signs in cervids

78. The clinical course of the disease may last only a few days or as long as a year or even longer (Kahn *et al.*, 2004; Wild *et al.*, 2002). In some deer the course of the disease is acute,

but generally it is longer, lasting typically a few weeks to a few (less than four) months (Williams *et al.*, 2002; Miller & Williams, 2004). It is probable that the disease in free-ranging cervids is more acute than that in captive individuals, since free-living individuals must forage and find water, and are also susceptible to predation (Miller, 1994, Williams *et al.*, 2002).

79. The main clinical signs of CWD in both *Odocoileus* spp. deer and in *Cervus elaphus nelsoni* are loss of body condition and changes in behaviour. The clinical signs may be more subtle in elk than in *Odocoileus* spp., and the clinical course may be longer (Williams & Miller, 2002).

80. In captive animals, increased or decreased interactions with handlers, repetitive behaviours (e.g. walking in a set pattern within the accommodation), lowered head and ear carriage and periods of somnolence or depression, from which the animal is easily roused, may be noted. Although affected animals continue to eat, they eat less than do their unaffected counterparts, resulting in a loss of body condition. Later, polyuria and polydipsia, increased salivation and associated drooling of saliva, and various nervous signs - incoordination, posterior ataxia, wide-based stance and fine head tremors - may be noted. Occasional signs include dilatation of the oesophagus, hyperexcitability and syncope (Williams & Miller, 2002).

81. In herds which have not previously been affected by CWD, the first cases may present clinically as prime age animals with aspiration pneumonia, which is probably due to difficulties in swallowing, and is unresponsive to treatment. Other presentation have included “sudden death” of animals after handling and unusual losses due to trauma (head stuck in fence) (Williams & Miller, 2002).

Clinical signs in *Cervus elaphus nelsoni*

82. In Rocky Mountain elk (*Cervus elaphus nelsoni*), the primary sign is often a progressive loss of body condition, sometimes seen as a failure to regain weight after the normal seasonal decrease in body weight (Williams & Young, 1982; Williams & Young, 1992; Miller, Wild & Williams, 1998). In captivity, affected animals have been noted to fail to regain weight even when provided high quality feed *ad libitum* (Miller, Wild & Williams, 1998).

83. Behavioural changes are variable and may include personality changes, changes in behaviour towards handlers, nervousness, hyperexcitability and hyperaesthesia. Occasional subtle abnormal head postures and/or hind limb ataxia have been noted. Excessive salivation and tooth-grinding are relatively common while polydipsia and associated polyuria are less pronounced than in *Odocoileus* spp. deer (Williams & Young, 1982; Williams & Young, 1992; Liberski *et al.*, 1993).

Clinical signs in *Odocoileus hemionus*

84. In mule deer (*Odocoileus hemionus*), CWD is generally seen as an insidious-onset chronic disease with weight loss and behavioural changes, progressing to emaciation and death (Williams & Young, 1980). Death occurs due to aspiration pneumonia, dehydration or hypothermia associated with cold weather if animals are not euthanased (Williams & Young, 1992). Slow weight loss occurs over weeks to months, progressing to emaciation (Williams & Young, 1992, Williams *et al.*, 1992, Liberski *et al.*, 1993). The hair coat is often poor and there may be failure to shed the coat normally (Williams & Young, 1992).

85. Excessive salivation, polydipsia/polyuria and terminal anorexia may be noted (Williams & Young, 1980, Liberski *et al.*, 1993). Tooth grinding was recorded in about 50% of affected mule deer in the initial description while oesophageal dilatation was seen in a few cases (Williams & Young, 1980). It has been suggested that polydipsia associated with polyuria, low urine specific gravity and dehydration may be related to lesions in the supraoptic and paraventricular nuclei (Williams & Young, 1993).

86. Behavioural changes and other neurological signs are initially subtle and vary from case to case; they may include decreased interactions with other deer, changes in responses to attendants, listlessness, drooping ears, lowered head, blank facial expression, periods of somnolence (from which the deer is easily roused) and repetitive walking in set patterns. In only a few individuals have hyperexcitability and hind limb ataxia been noted. Eventually there is increased depression and somnolence, finally recumbence (Williams & Young, 1992; Liberski *et al.*, 1993). Secondary aspiration pneumonia occurs terminally in some individuals (Williams & Young, 1992).

Clinical signs in *Odocoileus virginianus*

87. In white-tailed deer (*Odocoileus virginianus*), clinical signs of CWD include loss of weight and body condition and a poor hair coat. Excessive salivation and drooling, particularly in the terminal stages, often lead to wetting of the hair of the chin and neck. Food intake gradually diminishes; polydipsia and polyuria occur in most individuals, particularly in the terminal stages of the disease. Affected individuals may be found near water for prolonged periods. Behavioural changes, initially subtle, include listlessness and depression, decreased interaction with conspecifics and changed interaction with humans – there may be a loss of fear of humans. The head is often held low, ears drooping and subtle head tremors may occur. Other neurological signs may include subtle ataxia, incoordination and wide based stance (Williams *et al.*, 2002; Williams, Miller & Thorne, 2002; E.S. Williams, pers. comm., 2004). In the end-stage, affected individuals are emaciated and show pronounced behavioural changes, ataxia, and prominent drooling (Miller & Wild, 2004). Acute death occurs in some individuals due to aspiration (Williams *et al.*, 2002; Williams, Miller & Thorne, 2002; E.S. Williams, pers. comm., 2004). Cases with acute onset neurological signs (e.g. opisthotonus) and rapid death occur also (Miller & Wild, 2004).

88. No antibody response occurs in CWD (Williams, Kirkwood & Miller, 2001; Williams & Miller, 2002). The only consistent clinicopathological finding is reduced urine specific gravity, considered to be associated with polyuria/polydipsia (Williams & Young, 1980; Williams & Young, 1982; Williams & Young 1992).

Incubation period in cervids

89. The precise natural incubation period for CWD in cervids is not known (O'Rourke *et al.*, 2004; Spraker *et al.*, 2004). There are obvious difficulties in determining the date at which an individual became naturally infected, while the date of onset of clinical disease, which may be insidious, generally cannot be stated with certainty.

90. It is probably that incubation period varies with the frequency of exposure to the CWD agent and the dose of agent received (Spraker, 2003).

91. During depopulation of infected premises in Canada, cervids less than 12 months old have been confirmed CWD-positive by immunohistochemistry (Kahn *et al.*, 2004). In early studies it was noted that the youngest age at which deer became clinically affected was 18 months, suggesting this as a minimum incubation period (Williams & Young, 1992).

Canadian veterinary services consider the incubation period to be 16 to 36 months with a mean of 22 months (Salman, 2003).

92. In Rocky Mountain elk, the youngest recorded affected free-ranging elk was 21 months old (Spraker *et al.*, 1997); clinical CWD in farmed elk has been seen at 24 months (Ball, 2002). From epidemiological studies on two outbreaks in elk at a wildlife research facility in Colorado the estimated incubation period was 18 to 36 months (Miller, Wild & Williams, 1998). Following oral inoculation the observed incubation period was approximately 12 to 34 months (Williams & Miller, 2002; Williams, Miller & Thorne, 2002).

93. In mule deer, initial data from experimental intracranial inoculation gave incubation periods of 17 and 21.5 months (Williams, E.S., Young, S. & Marsh, R.F., 1982; Williams & Young, 1992). In later experiments using oral inoculation, the minimum incubation period from exposure to first clinical signs was about 15 months while the mean time from oral exposure to death was 23 months with a range of 20-25 months (Williams & Miller, 2002; Williams, Miller & Thorne, 2002; Williams *et al.*, 2002). It was noted that the time at which the earliest clinical signs were noted coincided with when lesions of spongiform encephalopathy were first detected in the brain (Williams & Miller, 2000). A study of naturally-infected free-living individuals suggested that the progression of disease may be more rapid for individuals infected early in life (Spraker *et al.*, 2002b).

94. In white-tailed deer free-living in Wisconsin, the youngest animal with clinical signs was about 1.5 years old; the youngest age at which infection was confirmed was about five to six months (J. Langenberg, pers. comm., 2003).

Morbidity/mortality in cervids

95. All known CWD affected cervids have either died or required euthanasia due to clinical signs; CWD is considered to be invariably fatal once clinical signs have developed (Williams & Young, 1980; Williams & Nile, 1985; Williams & Young, 1992; Williams & Miller, 2002; Williams, Miller & Thorne, 2002).

96. The annual incidence in the affected research facility cervids varied depending on the number of deer being maintained long-term (more than two years) in the facilities; during 1970 to 1981 about 90% of the mule deer resident for two years or longer developed CWD (about 60 deer). All affected animals died or were euthanased; morbidity and mortality at other infected facilities were noted to be similar (Williams & Young, 1992). In the herd of elk newly established in June 1986 at the Foothills Wildlife Research Facility in Colorado, after attempted eradication of CWD, CWD was the only natural cause of mortality in adult elk at the facility between June 1986 and May 1997. Four of the 23 elk (17%) that remained in the facility for more than 15 months died of CWD; five of 14 elk (36%) remaining more than 12 months in the previous herd at this site had also developed CWD (Miller, Wild & Williams, 1998).

97. It is recognised that a few individuals in research facilities with endemic CWD did not develop CWD during their lifetime, but the reasons for this, genetic or otherwise, are not known (Williams *et al.*, 2002).

Pathological findings

Gross pathology

98. The main gross pathological finding in deer and elk which have died of CWD or have been euthanased late in the course of the disease, is emaciation, with total loss of body fat, serous atrophy of fat and even severe muscular atrophy in terminal cases. The bone marrow

may be yellow and gelatinous (Williams & Young, 1980; Williams & Young, 1982; Spraker *et al.*, 1997; Williams & Young, 1992; Williams *et al.*, 1992; Spraker *et al.*, 1994). However some individuals die in fair body condition after a short clinical course or following death due to aspiration pneumonia (Williams & Miller, 2002).

99. In some individuals there are lesions of aspiration pneumonia, with or without fibrinous pleuritis (Williams & Young, 1980; Williams & Young, 1992; Williams *et al.*, 1992; Spraker *et al.*, 1997; Williams & Miller, 2002). Abnormal ruminal contents, watery or gassy, and often containing excessive amounts of sand or gravel, are more common in *Odocoileus* spp. deer than in elk (Williams & Young, 1980; Williams & Young, 1992; Williams *et al.*, 1992; Spraker *et al.*, 1997; Williams & Miller, 2002). A greatly dilated and fluid-filled oesophagus has been seen in a few mule deer, but not in any elk (Williams & Young, 1992). Marked enlargement of the adrenal glands has been noted in some mule deer (Spraker *et al.*, 1997).

100. There are no gross lesions of the central nervous system (Williams & Young, 1992).

Histopathology

101. Histologically, lesions of CWD are found within the CNS. The distribution of lesions is similar in all three natural host species (Williams & Miller, 2002). Qualitatively, lesions are similar to those seen with other TSEs; the topographical distribution of lesions, and the severity, are most similar to those of scrapie and BSE (Williams & Young, 1993).

102. Lesions are characterized by spongiform transformation (microcavitation) of the grey matter, neuronal intracytoplasmic vacuoles, loss and degeneration of neurons, hypertrophy and hyperplasia of astrocytes and absence of inflammatory response (Williams & Young, 1980; Williams & Young, 1982; Williams & Young, 1993; Spraker *et al.*, 1997; Spraker *et al.*, 2002c). In all individuals with clinical signs of CWD there are lesions in the olfactory tubercle and cortex, hypothalamus and parasympathetic vagal nucleus (dorsal motor nucleus of the vagus, DMNV, in the dorsal portion of the medulla oblongata at the obex) (Williams & Young, 1993; Williams & Miller, 2002). The first region of the brain to be IHC positive, and to develop lesions of spongiform encephalopathy, is the dorsal motor nucleus of the vagus (Spraker *et al.*, 2002b). Varying degrees of spongiform change may be present in other parts of the brain, particularly in the thalamus and the cerebellum; lesions in the cerebral cortex, hippocampus and basal ganglia are generally mild (Williams & Young, 1993; Williams & Miller, 2002). Lesions in some thalamic nuclei are more severe in elk than in deer (Williams & Young, 1993). White matter lesions are rarely seen in deer but mild lesions may be present in elk, usually rarefaction in the cerebrum and cerebellum, particularly in cerebellar subcortical regions (Williams & Young, 1993).

Electron microscopy

103. Ultrastructurally, in mule deer important findings included extensive membrane-bound vacuolation in neuronal processes, prominent astrocytic gliosis, dystrophic neurites, amyloid plaques and giant neuronal autophagic vacuoles. Findings were noted to be similar to those seen in other TSEs (Guiroy *et al.*, 1993a). In elk, findings included membrane-bound vacuoles, found only within neuronal elements, an increased number of glial filaments and the presence of dystrophic neuritis of varying size; there were numerous neuritic plaques as well as Hirano bodies and perikaryal inclusion bodies. It was noted that findings were similar to those seen in mule deer (Guiroy *et al.*, 1994).

104. Fibrils, resembling those seen in the brains of scrapie-infected hamsters, were described in the detergent extracts from brain tissues of *Cervus elaphus nelsoni* – Rocky

Mountain elk with CWD by Guiroy *et al.* (1993b). The fibrils were 10-20 nm in diameter and short, each consisting of one or two straight protofilaments, and could be found individually or in aggregates (Guiroy *et al.*, 1993b).

PrP deposition

105. Amyloid plaques composed of PrP^{CWD} can be seen in routinely stained (haematoxylin and eosin) brain tissue of most clinically-affected white tailed deer and in some mule deer but these are not obvious in brain sections from elk (Williams & Young, 1993; Liberski *et al.*, 2001; Williams & Miller, 2002). Plaques may be visualised more easily using special stains. They may be seen easily at low magnification using Bodian silver impregnation, are periodic acid-Schiff (PAS) positive and are also easily visualised with Congo red staining followed by viewing in polarized light; they are not visible in brains of elk even with such stains (Bahmanyar *et al.*, 1985; Guiroy *et al.*, 1991a; Williams & Young, 1993). Florid plaques, with a rim of spongiform vacuoles, were found in the brains of 13 of 16 mule deer in one study; they were found most commonly in the medulla and the basal ganglia and were rarest in the cerebral cortex. Other PrP^{CWD} deposits were perivascular or of the “punctate synaptic type” (Liberski *et al.*, 2001).

106. Immunohistochemical (IHC) staining for PrP using any of a range of polyclonal and monoclonal antibodies clearly reveals PrP^{CWD} plaques in the brains of affected animals. IHC may show granularity and amorphous clumps on neuronal membranes, perivascular aggregates of PrP^{CWD} and large accumulations of PrP^{CWD} which appear to be extracellular (Williams & Miller, 2002).

107. The first site in the brain at which PrP^{CWD} is detected is the dorsal motor nucleus of the vagus (DMNV) (Williams & Miller, 2000; Sigurdson *et al.*, 2001). In all individuals with clinical signs of CWD, there are lesions in the parasympathetic vagal nucleus in the dorsal portion of the medulla oblongata at the obex, and in the hypothalamus, thalamus, olfactory tracts and cortex (Williams & Miller, 2002).

108. In a study of naturally infected *Odocoileus hemionus* – mule deer showing clinical signs of CWD, PrP^{CWD} was detected by immunohistochemical staining in the medulla oblongata (abundant staining in the DMNV), intermediolateral cell column of the spinal cord, myenteric plexus, vagosympathetic trunk and nodose ganglion. Other nerves in which staining was seen in some individuals were the sciatic nerve (one of five deer), sympathetic trunk (one of four deer) and brachial plexus (one of four deer). No staining was found in the celiac ganglion, cranial cervical ganglion (only one individual sampled), gasserian ganglion, or dorsal and ventral spinal nerve roots. Additionally, PrP^{CWD} was detected in the pituitary (mainly the pars nervosa and pars intermedia) and often in the adrenal medulla (three of five deer) and pancreatic islets (in five of six deer, generally in less than 50% of islets, but abundant where present); it was suggested that this may reflect nerve-vectored transport of PrP^{CWD} to endocrine organs (Sigurdson *et al.*, 2001). Compared to the level of staining seen in the vagosympathetic trunk, that in the sciatic nerve and brachial plexus was scant. It was considered that the findings indicated transit of PrP^{CWD} in nerves (either centrifugally or centripetally) and that endocrine organs may be targets for accumulation of PrP^{CWD} (Sigurdson *et al.*, 2001). In another study (see paragraph 109) no IHC staining was found in the brachial plexus, vagus, sciatic nerve or sympathetic trunk of mule deer (Spraker *et al.*, 2002c).

109. In another study of captive and free-ranging mule deer with clinical CWD, besides the CNS, PrP^{CWD} was detected in the eyes (layer of optic nerve fibres, ganglion cell layer, inner and outer plexiform layers of the retina), in a wide variety of gastro-intestinal tract associated

and peripheral lymph nodes (tonsil, retropharyngeal, parotid, mandibular, abomasal, ruminal, mesenteric, ileocaecocolic, inguinal, prescapular and popliteal), Peyer's patches, lymphoid follicles of the colon, lymphoid follicles in the posterior nasal septum, spleen and thymus (Spraker *et al.*, 2002c). PrP^{CWD} was **not** detected in the peripheral nervous system (including the dorsal root ganglia, anterior mesenteric ganglion, trigeminal ganglion, brachial plexus, vagus, sciatic nerve or sympathetic trunk), bone marrow, salivary glands, tongue, digestive tract (non-lymphoid components) from oesophagus to colon, myenteric plexus, acinar or islet cells of the pancreas, liver, non-lymphoid components of the respiratory tract, heart, arteries, veins, endocrine glands (thyroid, adrenal, pars anterior of the pituitary), skeletal muscle, smooth muscle, urogenital tract (kidney, urinary bladder, ovary, uterus, placentomes, testis, epididymis), skin and subcutaneous tissues, nor in tissues from major organs of the one fetus tested (Spraker *et al.*, 2002c).

110. No information is available on relative levels of infectivity in tissues from bioassays, because of the relative resistance of mice to CWD (Sigurdson *et al.*, 1999). The recent development of transgenic mice (expressing cervid PrP) highly susceptible to CWD (Browning *et al.*, 2004; Kong *et al.*, 2004), and use of ferrets (Sigurdson *et al.*, 2003) may allow such work to be undertaken. Examination of tonsils from 48 CWD-positive mule deer *Odocoileus hemionus* using a monoclonal antibody (F99/97.6.1) dot-blot assay found that tonsillar PrP^{CWD} concentrations varied from 34 to 1,188 ng per 0.5 mg of initial wet weight of tissue (O'Rourke *et al.*, 2003).

Pathogenesis

Early lymphoid tropism

111. Oral transmission is considered likely to be the main route of natural transmission of CWD (Sigurdson *et al.*, 1999; Williams, 2001). Experimental studies using oral inoculation in *Odocoileus hemionus* – mule deer fawns showed that PrP^{CWD} could be detected first in lymphoid tissues which drained the oral and intestinal mucosa: the retropharyngeal lymph nodes, the tonsils, Peyer's patches from the ileum and ileocaecal lymph nodes (Sigurdson *et al.*, 1999). It was noted that this is very similar to the pattern of distribution seen in sheep naturally infected with scrapie. The cells in which PrP^{CWD} staining was seen were not randomly distributed but rather were found within germinal centres of lymphoid follicles; the pattern of staining was morphologically consistent with follicular dendritic cells. It was suggested that PrP^{CWD} might be taken up and propagated initially in the tonsils and the Peyer's patches of the ileum and move to the ileocaecal and retropharyngeal lymph nodes within dendritic cells emigrating to these sites via the lymphatics. However, staining was detected in the tonsils only at 78 and 80 days post inoculation (Sigurdson *et al.*, 1999).

112. Further studies (Langeveld *et al.*, 2002; Sigurdson *et al.*, 2002), using triple-label immunofluorescence and confocal microscopy, detected PrP^{CWD} mainly extracellularly associated with follicular dendritic cell (FDC) membranes and B-cell membranes, as indicated by co-localisation with membrane-bound immunoglobulin and CD21; co-localisation with cytoplasmic labels for FDC was minimal. Coarse intracytoplasmic aggregates of PrP^{CWD} were detected in scattered tingible body macrophages in the germinal centre, suggesting that PrP^{CWD} might be phagocytosed on FDC processes, apoptotic FDC or B-cells, or alternatively replication of PrP^{CWD} within macrophages. Cellular distribution of PrP^{CWD} was similar in asymptomatic naturally infected deer and in advanced disease. Very early in experimental oral infection PrP^{CWD} was detected mainly on membrane surfaces of FDC and B-cells, with no PrP^{CWD} apparent in tingible body macrophages of a fawn at 42 days post infection although PrP^{CWD} was found within macrophages in a fawn at 11 weeks post oral inoculation. It was postulated that FDC may convert PrP^{CWD} at the cell membrane or, if intracellular conversion

occurs, this is followed by rapid exocytosis of the PrP^{CWD}. The role of macrophages was not clear (Sigurdson *et al.*, 2002).

Changes in distribution of PrP and lesions with time during infection

113. Data from studies in naturally and experimentally infected *Odocoileus hemionus*, and naturally infected *Cervus elaphus nelsoni*, suggest that the parasympathetic region of the vagus nerve in the medulla may be the earliest site of PrP^{CWD} accumulation in the brain in the natural hosts of CWD (Peters *et al.*, 2000; Williams & Miller, 2000; Sigurdson *et al.*, 2001; Miller & Williams, 2002; Spraker *et al.*, 2002b; Spraker *et al.*, 2004).

114. When mule deer fawns of about five months old were orally inoculated, PrP^{CWD} was detected in cervical lymph nodes at three months post inoculation. PrP^{CWD} was widespread in lymphoid tissues by six months post inoculation, at which time PrP^{CWD} was detected in the CNS for the first time, in the medulla oblongata, at the lateral aspect of the parasympathetic vagal nucleus. Lesions of spongiform encephalopathy did not develop at this site until at least 15 or 16 months post inoculation, at which time early clinical signs were present (Williams & Miller, 2000; Williams & Miller, 2002).

115. One study, based on histopathology and detection of PrP^{CWD} by immunohistochemistry (using monoclonal antibody F89/160.1.5) in the brain and palatine tonsil of 35 hunter-killed free-living mule deer with natural CWD, suggested four categories of infection (Spraker *et al.*, 2002b):

Category 1: detectable PrP^{CWD} in the tonsil but no evidence of spongiform encephalopathy in the brain and no detectable PrP^{CWD} in the brain by IHC;

Category 2: positive by IHC in the tonsil and the dorsal motor nucleus of the vagus nerve (DMNV), with or without histological lesions of spongiform encephalopathy at this site;

Category 3: positive by IHC in the tonsil, positive by IHC with histological lesions of spongiform encephalopathy in the myelencephalon and PrP^{CWD} positive by IHC in the hypothalamus, but without lesions of spongiform encephalopathy in this region;

Category 4: positive by IHC in the tonsil, with the presence of PrP^{CWD} detectable by IHC throughout the brain and lesions of spongiform encephalopathy throughout the brain.

116. It was suggested that these categories may represent stages in the pathogenesis of CWD, with category one animals having early lymphoid tissue localisation of PrP^{CWD}, while categories two to four representing the progression of spread through the CNS (Spraker *et al.*, 2002b).

117. A study of 226 naturally infected Rocky Mountain elk similarly demonstrated that PrP could be detected in the lower half of the dorsal nucleus of the vagus nerve without any staining of other regions of the myelencephalon at the obex, or could be found in this nucleus plus other surrounding nuclei. Histopathological lesions were detected only in the sections from brains with relatively heavy PrP^{CWD} and widespread deposition (Spraker *et al.*, 2004).

Variations between species

118. The progression of infection in *Cervus elaphus nelsoni* differs somewhat from that in *Odocoileus* spp. deer (Williams *et al.*, 2002). As with deer, the parasympathetic region of the dorsal nucleus of the vagal nerve appears to be the first brain region to become infected; a study of more than 10,000 elk found that in some individuals PrP^{CWD} was detectable only at this site, not elsewhere in the brain (Spraker *et al.*, 2004). However, lymphoid tissue involvement, as indicated by detection of PrP^{CWD}, is more variable than in *Odocoileus* spp.

deer. In a study involving testing of more than 10,000 elk, in which 226 animals were CWD-positive, 155 (68.6%) had PrP^{CWD} deposits in both brainstem and lymphoid tissue, 43 animals (19%) were CWD positive only in lymphoid tissue. Within the lymphoid tissues, some animals had staining only in the retropharyngeal lymph node, some only in the tonsil and others in both lymphoid tissues; variation in whether and which lymphoid tissue was PrP^{CWD}-positive occurred for all recorded levels of brain infection (Spraker *et al.*, 2004).

Individual variation

119. Studies on both naturally infected *Odocoileus* spp. deer and individuals experimentally infected by oral inoculation indicate that lymphoid tissues become PrP^{CWD} positive before detectable involvement of the CNS (Sigurdson *et al.*, 1999; Spraker *et al.*, 2002b; Miller & Williams, 2002). However there may be some natural variation in pathogenesis; occasional individuals are detected in which the obex is positive for PrP^{CWD} by IHC but tonsil and/or retropharyngeal lymph node are negative (Spraker *et al.*, 2002a; Miller & Williams, 2002).

6) DIAGNOSIS

120. Clinical signs (loss of body condition and behavioural changes) are commonly used for detection of CWD in both captive and free-ranging cervids (Miller & Williams, 2004), however these signs are not pathognomonic and, as noted in paragraphs 78-81, the presentation of clinical cases may vary. Additionally, due to the progressive nature of the disease, casual inspection, even by experienced personnel, may fail to detect individuals with clinical CWD (Miller & Williams, 2004). A wide variety of diseases which cause emaciation and/or nervous signs must be included as differential diagnoses for cervids with CWD (Williams, Kirkwood & Miller, 2001; Williams & Miller, 2002).

121. Definitive diagnosis requires histopathological examination of the brain for spongiform lesions (Williams & Young 1980; Williams & Young, 1982; Williams & Young 1992, Williams & Young 1993) and/or immunohistochemical detection of PrP^{CWD} accumulation in the lymph nodes and/or brain (Williams & Young 1992; Miller *et al.*, 2000; Peters *et al.*, 2000, Spraker *et al.*, 2002b; Williams & Miller, 2002).

122. Several tests are now available for diagnosis of CWD in cervids. Tests should be validated for the tissue and species on which they should be used; additional factors which are considered when choosing diagnostic tests for large-scale CWD surveillance include the time required to process the samples, the quantity of samples which can be processed and the condition (e.g. fresh, decomposed) of the tissue to be tested (Samuel *et al.*, 2003).

123. It should be noted that whatever the sensitivity of the tests used, because of uncertainties regarding incubation time and the interval from natural infection to the appearance of detectable PrP^{CWD} in tested tissues, an individual cervid in which PrP^{CWD} is not detected cannot be guaranteed not to be infected with CWD (Wild *et al.*, 2002; Samuel *et al.*, 2003; Spraker *et al.*, 2004).

Histopathology

124. Initially, the presence of typical lesions of spongiform encephalopathy (microcavitation of the grey matter, neuronal intracytoplasmic vacuoles, loss and degeneration of neurons, astrocytosis) in the olfactory tubercle and cortex, hypothalamus and the parasympathetic vagal nucleus were recognised as sufficiently consistent that histopathological examination of these areas could be used for diagnosis. It was noted that in healthy (CWD-negative) individuals a few intraneuronal vacuoles may be found in the red nucleus, but not in other sites (Williams & Young, 1992; Williams & Young, 1993).

125. For several years it has been recognised that the dorsal motor nucleus of the vagus (DMNV), in the dorsal part of the medulla oblongata at the obex, is the earliest and most consistent site in the brain at which histopathological lesions of CWD can be detected in all three known natural hosts of CWD (*Cervus elaphus nelsoni*, *Odocoileus hemionus* and *Odocoileus virginianus*) (Peters *et al.*, 2000; Miller *et al.*, 2000; Williams & Miller, 2000; Spraker *et al.*, 2002b; Miller & Williams, 2004).

126. Disadvantages of histopathology for diagnosis include the requirements for very fresh tissues (no autolysis), which are not always available from field specimens (Spraker *et al.*, 2002a; Kahn *et al.*, 2004) and the fact that histological lesions do not develop until quite late in the course of infection (Miller & Williams, 2002; Spraker, 2003) (see paragraphs 114-116).

Immunohistochemistry

127. Immunohistochemical (IHC) staining for PrP^{CWD} has been used to confirm diagnosis of CWD for some time (Williams & Young, 1992). PrP^{CWD} can be detected by IHC in brain tissue and/or lymphoid tissue in the absence of histopathological spongiform lesions (Sigurdson *et al.*, 1999; Peters *et al.*, 2000). IHC of the parasympathetic vagal nucleus in the dorsal medulla at the obex is sensitive and specific for CWD diagnosis in *Odocoileus* spp. deer and in *Cervus elaphus nelsoni* (Miller *et al.*, 2000; Miller & Williams, 2002; Miller & Williams, 2004). IHC has high sensitivity and high specificity (Creekmore, 2003; Bollinger *et al.*, 2004). IHC, particularly using monoclonal antibody F99/97.6.1, is still considered by most working in the CWD field to be the “gold standard” (Creekmore, 2003; Samuel *et al.*, 2003; Hall, 2004; Kahn *et al.*, 2004); it allows precise anatomical considerations of PrP deposition in addition to the simple presence of PrP^{CWD} (Creekmore, 2003), and is considered not to produce any false positive reactions (Hall, 2004).

128. Monoclonal antibodies used for IHC do not in themselves differentiate between the normal cellular PrP^C and the disease-associated form of PrP. Specificity for the disease-associated form therefore requires appropriate pretreatment of the tissue to denature PrP^C (Spraker *et al.*, 2002a; Williams & Miller, 2002).

129. One advantage of IHC over histopathological examination using standard stains such as haematoxylin and eosin is that it can be used on tissue which is somewhat autolysed (Spraker *et al.*, 2002a).

130. Several monoclonal antibodies have been evaluated for use in CWD diagnosis. F99/97.6.1 (O’Rourke *et al.*, 2000) has been proven as the most reliable for use on brain, retropharyngeal lymph node or tonsil and is the monoclonal antibody used most commonly for IHC in the USA and Canada (Miller & Williams, 2000; Spraker *et al.*, 2002a; Samuel *et al.*, 2003; Spraker, 2003; Miller & Williams, 2004). F99/97.6.1 reacts with a conserved epitope, residues QYQRES) found on the prion protein of mule deer, white-tailed deer and Rocky Mountain elk as well as sheep, cattle, mink and various other species (O’Rourke *et al.*, 2000; Spraker *et al.*, 2002a; Spraker, 2003). This antibody was validated on brain and tonsil samples from 100 CWD-positive and 300 CWD-negative mule deer and was found to be excellent for detection, including for use on fresh or frozen tissues and on tissues which were mildly to moderately autolysed (Spraker *et al.*, 2002a). It was noted that Mab F89/160.1.5 was also highly specific for deer brain but gave some false-positive reactions in deer tonsils, probably due to detection of the epitope on PrP^C (Spraker *et al.*, 2002a).

131. Lymphoid tissues, specifically the tonsils and the retropharyngeal lymph nodes, can also be used for diagnosis of CWD using IHC in *Odocoileus* spp. deer (Spraker *et al.*, 2002a;

Miller & Williams, 2002; Miller & Williams, 2004). These tissues are easily collected from the heads of hunter-harvested deer (Williams *et al.*, 2002; Miller & Williams, 2004).

132. In most *Odocoileus* spp. deer, these tissues are involved relatively early in infection (Sigurdson *et al.*, 1999; Spraker *et al.*, 2002b; Miller & Williams, 2002), therefore IHC of tonsil and/or retropharyngeal lymph node alone (i.e., without also testing the brain stem at the obex), may be sufficient for epidemiological surveys of prevalence in wild deer (Spraker *et al.*, 2002a; Miller & Williams, 2002). However, due to variations between species, and individual variation, it is recommended that to maximise detection of CWD infected individuals both the obex and cranial lymphoid tissues should be tested for the presence of PrP^{CWD} (Williams *et al.*, 2002; Miller & Williams, 2004). Further information on these differences is provided in paragraphs 118-119. Additionally it must be remembered that failure to detect PrP^{CWD} in these tissues does not totally rule out the possibility that the individual is infected; a negative result may occur early in the course of infection (Sigurdson *et al.*, 1999; Miller & Williams, 2002; Wild *et al.*, 2002; Samuel *et al.*, 2003).

133. In *Odocoileus* spp. deer, IHC can also be used to detect PrP^{CWD} in biopsy specimens from the tonsils of live individuals. This procedure requires the deer to be anaesthetised, so is not suitable for large-scale surveillance operations, however it may be useful in situations where harvest-based surveillance is not practical and where intensive management is feasible (e.g. deer resident in urban areas, or in national parks) (Wild *et al.*, 2002; Wolfe *et al.*, 2002; Wolfe, Miller & Williams, 2004). Diagnosis from tonsillar biopsies is not applicable to *Cervus elaphus nelsoni* because of the differences in PrP^{CWD} accumulation in lymphoid tissues in this species (Wild *et al.*, 2002; Spraker, 2003; Spraker *et al.*, 2004). Sampling of conjunctival lymphoid tissue, as used in sheep (O'Rourke *et al.*, 1998b; O'Rourke *et al.*, 2000), is not applicable to deer because of the sparsity of this tissue in deer (Williams & Miller, 2002; Kahn *et al.*, 2004).

134. Potential disadvantages of immunohistochemistry, particularly for large-scale surveillance programmes, are that it takes time (including the delay in preparing tissues prior to reading the slide), requires specialised skills, it is subjective (as opposed to tests utilising an objective numerical cut-off point to separate negative from positive samples), and it may potentially be influenced by prior knowledge of the case from which the sample was taken (including the results of other tests carried out on the sample) (Salman & Gardner, 2004).

135. Where the new "rapid" tests are used for surveillance, IHC is recommended as a secondary test to confirm positives detected by the initial screening test and to reject false positives (Samuel *et al.*, 2003; Bollinger *et al.*, 2004).

Electron microscopy

136. Negative stain electron microscopy to detect scrapie-associated fibrils (SAF) (see paragraph 104) can be used as a supplemental diagnostic test for CWD in mule deer, white-tailed deer and elk (Williams & Young, 1992; Spraker *et al.*, 1997; Williams & Miller, 2002). SAF may be found in the spleen as well as in the brain (Spraker *et al.*, 1997; Williams & Miller, 2002). As with immunohistochemistry, SAF can be demonstrated even in autolysed tissues (Williams & Young, 1992).

Other tests

137. New "rapid" tests for CWD (ELISA and Western blot) are now being used. Commercially available tests are based on those developed for the detection of BSE. These tests use unfixed tissues (Bollinger *et al.*, 2004), which removes a delay in time taken from sampling to test results. As with IHC the sensitivity of such tests depends on the tissue(s)

being tested. In general, these tests have a high sensitivity but a lower specificity, leading to some false-positive results (Bollinger, 2004).

ELISA

138. ELISA-based test kits have the advantage that they allow rapid testing of large numbers of samples (Creekmore, 2003). ELISA-based test kits from three different companies have been approved by USDA Center for Veterinary Biologicals for use as screening tests for the detection of CWD in free-ranging cervid populations, but none has yet been approved for use for testing of farmed cervids in regulatory programmes. One test kit, from VMRD, has since been withdrawn from the market.

139. A Bio-Rad test kit was approved by APHIS for use in mule deer (*Odocoileus hemionus*), white tailed deer (*Odocoileus virginianus* - White-tailed deer) and elk *Cervus elaphus nelsoni* - Rocky Mountain Elk by testing specific lymph nodes (APHIS, 2002, B.L. Morrison, pers. comm., 2003). According to the manufacturer, the Bio-Rad test “*combines a purification protocol for increased sensitivity with rapid detection by ELISA. Results are available in 4 hours.*” Two specific monoclonal antibodies are used in the test (Bio-Rad, 2004). The Bio-Rad ELISA CWD antigen test kit was validated using 4,175 retropharyngeal lymph node or obex samples, with the results of the ELISA being compared with immunohistochemistry (IHC) findings (IHC-positive individuals being taken as being CWD-infected and IHC-negative individuals being taken as uninfected) (Hibler *et al.*, 2003). The relative specificity of the ELISA was 99.9-100% and the sensitivity was 98.3-100% for retropharyngeal lymph node samples and 92.1-93.3% sensitive for obex samples. Overall there was agreement for at least 97.6% of lymph node and at least 95.7% of obex samples where values could be calculated. ELISA optical density (OD) values were at least 46 times higher for IHC-positive than for IHC-negative samples. Discrepancies between IHC and ELISA results were found only for early-stage CWD cases. In the field-application stage, 20,875 retropharyngeal lymph node samples were screened with the ELISA. The 155 of 8,877 mule deer, 33 of 11,731 elk and nine of 267 white-tailed deer with ELISA OD values greater than 0.1 (value based on data from the validation phase of the study) were classified as “CWD-suspect” and evaluated by IHC; 143 of 155 mule deer, 29 of /33 elk and all nine white-tailed deer were IHC-positive and mean ELISA OD values were comparable to those measured during the validation stage. It was considered that the Bio-Rad ELISA was “*an excellent rapid test for screening large numbers of samples in surveys designed to detect CWD infection in deer and elk populations.*” From the study an OD cut-off of greater than or equal to 0.1 was recommended for screening retropharyngeal lymph node tissues of deer and elk in large-scale surveys to minimise the risk of false-negative results, giving a screening test with about 99.6% sensitivity, with a follow-up of IHC, carried out by a reliable experienced laboratory, to remain as the final determination test for CWD on ELISA-positive samples (Hibler *et al.*, 2003). A second Bio-Rad ELISA-based test (TeSeE®) was approved by USDA during 2003 (Chronic Wasting Disease Alliance, 2003).

140. The HerdChek® test kit from IDEXX also has been approved by USDA (IDEXX, 2004a). The HerdChek test uses homogenised lymph node tissue. The process takes a total of three-and-a-half hours and “*offers 98.8% sensitivity and 100% specificity, validated through IHC confirmation testing.*” Sensitivity was 98.8% (80 of 81 samples) and specificity 100% (248 samples) as confirmed by IHC on samples from *Odocoileus virginianus* - White-tailed deer (IDEXX, 2004b; IDEXX, 2004c).

Immunoblotting

141. Immunoblotting techniques are carried out on fresh or frozen, but not fixed, tissues (Stack, Keyes & Scott, 1996).

142. **Western blot** assays may be used for detection of PrP^{CWD} in the brains of both clinically affected and (presumably) preclinical cervids (Laplanche *et al.*, 1999). In a study on more than 10,000 *Cervus elaphus nelsoni*, tissue for Western blotting was taken from the medulla caudal to the section used for IHC. No samples which were negative by IHC were positive by Western blot. While all samples from 46 elk with heavy PrP^{CWD} deposits in the dorsal nucleus of the vagus and spreading to surrounding nuclei were positive on the Western blot, only 16 of 30 samples from elk with only moderate PrP^{CWD} deposits restricted to the dorsal nucleus of the vagus nerve and the solitary tract, and only five of 27 samples from elk with only scant PrP^{CWD} deposits restricted to the dorsal nucleus of the vagus nerve and the nucleus of the solitary tract, were positive. The discrepancies were probably due to lack of the dorsal motor nucleus of the vagus nerve within the tissue sample used for the Western blot assay (Spraker *et al.*, 2004). Western blotting was also carried out on lymphoid (tonsil and retropharyngeal lymph node) tissues; in 33 elk this test detected PrP^{CWD} deposits in the brain and lymphoid tissue, in three it detected PrP^{CWD} in the brain but not in lymphoid tissue, and in two it detected PrP^{CWD} in lymphoid tissue but not in the brain (Spraker *et al.*, 2004).

143. A **dot-blot assay** using monoclonal antibody F99/97.6.1 has been described using 150 mg of frozen tonsillar tissue in a 10% (weight/volume) detergent lysate without either purification or enrichment steps in the preparation. The assay detected PrP^{CWD} in 49 of 50 tonsils which were positive by IHC. Poor trimming (i.e. lack of relevant tonsillar tissue in the tissue prepared for dot-blot) was considered the most likely reason for the single discrepant result; the IHC was carried out on the central portion of the original sample while the dot-blot was carried out on one of the two flanking pieces of tissue. Quantification was possible from 48 of the samples. This was based on densitometry readings of each blot, extrapolated against a standard curve for each filter based on known quantities of ovine PrP^{Sc} sharing the conserved epitope for Mab F99/97.61, with mean density of the signal for each calibrator plotted against concentration for linear regression analysis. Tonsillar PrP^{CWD} concentrations were found to vary from 34 to 1,188 ng per 0.5 mg of initial wet weight of tissue (O'Rourke *et al.*, 2003).

Conformation-dependent immunoassay (CDI)

144. The **conformation-dependent immunoassay (CDI)** has been tested for the detection of CWD prions and found to have a sensitivity comparable with transgenic mouse bioassays for BSE (Safar *et al.*, 2002). Using this test, which depends on the antibody binding affinity to PrP^{Sc} conformers and is PrP^{Sc} strain-specific, samples from *Odocoileus virginianus* – white-tailed deer and *Odocoileus hemionus* – mule deer were found to be similar to one another. However, samples from *Cervus elaphus nelsoni* – Rocky Mountain elk could be differentiated from those of the other two species. The finding was considered to indicate a difference in conformation between PrP^{Sc} from *Odocoileus* spp. deer and that from the elk, but it was not possible to say whether this indicated a prion strain difference (Safar *et al.*, 2002).

Limitations of rapid tests

145. In a few cases, IHC may be more sensitive than other tests by virtue of detection in specimens with only occasional single-cell staining. In most samples IHC shows immunostaining of more than half the lymphoid follicles within a lymphoid tissue biopsy specimen, and in such tissues the rapid tests should also detect the PrP^{CWD} (O'Rourke *et al.*, 2003).

146. An important limitation of the rapid tests is a lack of certainty regarding the precise identity of the tissue being tested. While absence of correct tissue may also affect results of testing by IHC, the architecture of the tissue is preserved for IHC therefore it is possible to

identify whether or not the required area is present in the sample. It has been noted that standardisation of both tissue collection and tissue trimming techniques are essential for accuracy of high-throughput tests (Hibler *et al.*, 2003; O'Rourke *et al.*, 2003). A study using the CDI noted that measured PrP^{Sc} concentrations within sections of the obex region in cattle could vary markedly (eight-fold) depending on exactly which part of the obex was sampled. This finding confirms the importance of consistency of samples used in the test (Safar *et al.*, 2002).

147. While rapid tests are often used for screening of large numbers of samples in surveillance programmes, positive results are confirmed using IHC. This is particularly important for confirming the presence of CWD in a geographic location in which it has not previously been detected (Samuel *et al.*, 2003).

Choice of tissues for testing

148. As with IHC, testing of lymph nodes or tonsils will not detect individuals very early in the course of infection (Sigurdson *et al.*, 1999; Miller & Williams, 2002; Wild *et al.*, 2002). Testing these tissues only, not obex also, will fail to detect those individuals in which PrP^{CWD} has not accumulated in lymphoid tissue, but is present in the brain at the obex. This situation is rare for *Odocoileus* spp. deer but relatively common in *Cervus elaphus nelson* (Miller & Williams, 2002; Wild *et al.*, 2002; Spraker, 2003; Spraker *et al.*, 2004).

7) EPIDEMIOLOGY AND TRANSMISSION

149. Knowledge of CWD transmission and epidemiology is incomplete (Samuel *et al.*, 2003). CWD is thought to be spread mainly by lateral transmission. Some cases have occurred in offspring of CWD-affected dams; it has not been possible to prove whether transmission of infection in such cases was maternal or lateral (Williams & Young, 1992). A mathematical model of CWD transmission could not rule out a maternal transmission component but did indicate that maternal transmission alone would not allow the disease to persist (Miller *et al.*, 2000).

150. Observations in Canada also support the theory that lateral transmission is important in CWD epidemiology. In several herds it appears that a single infected cervid was introduced and the disease was then transmitted laterally to other cervids within the herd (unpublished data cited by Kahn *et al.*, 2004).

151. It is probable that interspecies transmission occurs between the natural hosts; suspected transmission has been observed from mule deer to elk and to white-tailed deer and from elk to both mule deer and white-tailed deer (Williams & Miller, 2002; Williams, Miller, & Thorne, 2002).

152. Disease may be transmitted between captive and free-living populations (Samuel *et al.*, 2003). It is possible that transmission of CWD may occur between free-living and captive cervids, in either direction, by prolonged fence-line contact (Williams & Young, 1992; Williams *et al.*, 2002).

153. It should be remembered that the route of "shedding" of the CWD agent is unknown, it is not known how long after infection shedding of agent first occurs, and nor is the minimum infectious dose of agent known (Creekmore, 2003). The most likely routes of transmission are via saliva, urine and faeces (Bollinger *et al.*, 2004). Transmission by contact between infected and non-infected animals probably requires longer than just transient exposure (Bollinger *et al.*, 2004).

Potential sources and transmission routes

154. It is not known at what point during the course of infection cervids become infectious, however it appears likely that shedding of the agent is progressive through the course of the disease, and that shedding starts before the onset of clinical signs (Williams & Miller, 2002; Van Deelen, 2003; Bollinger *et al.*, 2004).

155. Tissues in which PrP^{CWD} has been detected by IHC and which may therefore act as sources of PrP^{CWD} include the brain and retina of mule deer, lymphoid tissues (gut-associated lymphoid tissues, mesenteric lymph nodes, lymphoid follicles in the posterior nasal septum, tonsils, visceral and peripheral lymph nodes and Peyer's patches) (Spraker *et al.*, 2002c), and in the pituitary (pars intermedia and pars nervosa), the adrenals (adrenal medulla) and the pancreas (islets of Langerhans) (Sigurdson *et al.*, 2001). Tissues in which PrP^{CWD} has **not** been detected by IHC and which are therefore unlikely to act as sources of PrP^{CWD} include the peripheral nervous system (including the dorsal root ganglia, anterior mesenteric ganglia and trigeminal ganglia), parotid and mandibular salivary glands, tongue, oesophagus, the digestive tract rumen, abomasum, small intestines and colon except for gut-associated lymphoid tissues, respiratory system (trachea, bronchi, bronchioles, alveolar parenchyma) except for lymphoid follicles in the posterior nasal septum, cardiovascular system (myocardium, Purkinje fibres, walls of peripheral arteries and veins), thyroid, pars anterior of the pituitary, musculoskeletal system (skeletal muscle), smooth muscle, and urogenital system (kidney, urinary bladder, ovary, uterus, placentomes, testis, epididymus), and skin (including epidermis, dermis, and subcutaneous tissues including sebaceous, sweat, lachrymal and tarsal glands) (Spraker *et al.*, 2002c).

156. Given the presence of PrP in lymphoid tissues associated with the alimentary tract early in the course of disease it seems possible that shedding of the agent may occur at an early stage of infection. Excretion of the agent in faeces is plausible and if the agent is present in saliva then this also may be a source of agent; in the terminal stages of CWD affected animals drool saliva (Williams & Miller, 2003); nasal secretions may also be involved (Wild & Miller, 2004). Shedding of PrP^{CWD} is probably progressive during the course of infection (Miller *et al.*, 2000). From epidemic models it is probable that shedding of PrP^{CWD} precedes the onset of clinical disease in an infected cervid (Williams, Miller, & Thorne, 2002). Epidemic models of captive mule deer suggest that shedding may occur from about half way through the total incubation period and models assuming shedding about 12 to 18 months after infection have represented the dynamics which have been observed for CWD in free ranging mule deer (Williams & Miller, 2003). Additionally, following the death of an infected individual, environmental contamination with the agent could occur by scavenging (including by invertebrates) and decomposition (Williams & Miller, 2003).

157. Transmission of CWD probably occurs by both direct and indirect routes (Williams & Miller, 2002). Since TSE agents are very resistant in the environment, indirect transmission may occur via environmental contamination (Williams *et al.*, 2002). Environmental contamination has been proven experimentally to be able to act as a source of infection (see paragraph 162)(Miller *et al.*, 2004) and appears to play a role in the maintenance of CWD, at least in captive populations, as has been indicated by the recurrence of CWD in facilities following complete depopulation and subsequent repopulation, even when repopulation was carried out from a certified CWD-free herd (Woodbury, 2001).

158. The possibility of an unidentified invertebrate reservoir of infectious agent, such as hay mites, in the environment, has been considered (Miller, Wild & Williams, 1998; Salman, 2003); no data is yet available. The possibility of an unidentified vertebrate reservoir should also be considered (Salman, 2003).

159. It is also not possible to discount the possibility that prion-contaminated feed may initiate a CWD epidemic (Miller & Williams, 2004).

Experimental data on natural transmission routes (i.e. not including inoculation)

Contact

160. Mule deer (*Odocoileus hemionus*) from CWD-negative source populations, maintained in paddocks together with a known naturally CWD-infected mule deer (one infected deer per test paddock), became infected (in two of three replicate paddocks) within about one year. Infection in the experimental animals was detected initially by immunohistochemical staining using anti-PrP monoclonal antibody (Mab) 99/97.6.1 and then confirmed. Two of ten deer became infected in this experiment: one of four deer in each of two paddocks but neither of two deer in the third paddock (Miller *et al.*, 2004).

From enclosures previously used by infected animals

161. It has been recognised for some time that environmental contamination may play a significant role in the transmission of CWD; contaminated pastures appear to have acted as sources of the agent in some epidemics of CWD (Miller *et al.*, 2000; Williams *et al.*, 2002).

162. Mule deer *Odocoileus hemionus* from CWD-negative source populations, maintained in paddocks which had been empty for about 2.2 years but had previously held orally-inoculated mule deer and had not been cleaned of residual excreta (about 3.8 infected mule deer x years of excreta per paddock), became infected (in one of three replicate paddocks) within about one year. Infection in the experimental animals was detected initially by immunohistochemical staining using anti-PrP monoclonal antibody (Mab) 99/97.6.1 and then confirmed. One of nine deer became infected in this experiment: one of three deer in one paddock but none of three deer in either of the other two paddocks. It was noted that the presence of PrP^{CWD} in gut-associated lymphoid tissue of infected mule deer implicates alimentary shedding of the agent in both faeces and saliva. While the exact mechanism of CWD transmission in these paddocks could not be determined, the most plausible options were suggested to be foraging and ingestion of soil (Miller *et al.*, 2004). It was noted that since the infectivity in the paddocks could not be measured it was not possible to give information on either the initial levels of infectivity or the degradation rate of the CWD agent in the environment (Miller *et al.*, 2004).

From carcasses

163. Mule deer *Odocoileus hemionus* from CWD-negative source populations, maintained in paddocks containing carcasses of infected mule deer became infected (in two of three replicate paddocks) within about one year. One carcass was provided per paddock, from a mule deer euthanased in end-stage clinical CWD, left intact (except for a small brainstem sample removed to confirm infection) and left to decompose *in situ* for about 1.8 years before the test deer were introduced into the paddock with the now skeletonised carcass. Infection in the experimental animals was detected initially by immunohistochemical staining using anti-PrP monoclonal antibody (Mab) 99/97.6.1 and then confirmed. Three of twelve deer became infected in this experiment: two of four deer in one paddock, one of five deer in one paddock and none of three deer in the third paddock. It was noted that while the deer did not actively consume decomposed remains of carcasses they did forage in the immediate vicinity of the carcasses and it was likely that a nutrient flush in this area produced lush vegetation. (Miller *et al.*, 2004).

Problems of eradication/environmental contamination

164. Two attempts have been made to eradicate CWD from infected research facilities. In a Wyoming facility all cervids in the main portion of the site, where CWD had occurred, were killed and animals were not reintroduced for a year; CWD recurred about five years later. No attempt had been made to disinfect or turn the soil (Williams & Young, 1992). Attempted elimination of infection at the Foothills Wildlife Research Facility, Fort Collins, Colorado (and its satellite facilities) involved killing all cervids residing in the facilities in 1985, treating paddocks in which affected cervids had resided by spraying with 1,000 ppm calcium hypochlorite solution (65% available chlorine), ploughing to about 0.3 m deep and re-spraying, replacing shelters, feed bunkers and automatic waterers or hand cleaning these twice with 1,000 ppm calcium hypochlorite solution, constructing an eight-foot high deer-proof perimeter fence and leaving the enclosures unoccupied for 12 months before restocking with wild-caught fawns in June 1986. The disease recurred after restocking (Neil, 1985; Neil, 1986; Murphy, 1994; Miller, Wild & Williams, 1998).

165. It is worth noting that while CWD has occurred in cervids in, probably, three zoos, the disease has apparently not been maintained in these locations: no further cases have occurred following the initial cases in cervids with links to infected research facilities in Colorado and Wyoming, despite the lack of any deliberate attempt to disinfect the areas. (Laplanch *et al.*, 1999; Williams *et al.*, 2002).

Epidemiology and spread in captive and farmed cervids

166. Spread of CWD between farms probably occurs by human movement of infected animals for commercial purposes (Miller & Williams, 2003). The potential for spread of CWD by this means has been recognised for some time (Williams & Young, 1992; Miller & Williams, 2004).

167. Within the captive elk herds of Canada it appears that all transmissions have been caused by movement of live elk between farms (Canadian Food Inspection Agency, 2002).

168. Given that lateral transmission, whether direct or indirect, is important, concentrating populations of cervids, as occurs in captivity, is likely to increase transmission (Williams, Miller & Thorne, 2002; Miller & Williams, 2003).

169. Lateral transmission from mule deer to elk is considered the most likely source of CWD in elk in research facilities in Colorado and Wyoming where the disease was seen in mule deer and then later in elk (Williams & Young, 1992). Lateral transmission between elk was indicated by the study of an outbreak in elk at the Foothills Wildlife Research Facility, Fort Collins, Colorado, in which the affected animals, brought into the facility from the wild and hand-reared, did not have any contact with other cervid species. It was acknowledged that common-source exposure, such as environmental contamination, could also have been involved (Miller, Wild & Williams, 1998).

170. Epidemiological studies of captive mule deer in research facilities indicate that, if maternal transmission occurs, it is relatively rare (Miller *et al.*, 2000; Miller & Williams, 2003). Epidemiological studies on captive elk in research facilities could not totally rule out the possibility of maternal transmission to calves prior to their being brought into the facility (since calves were brought in from a population later recognised to be CWD-positive), but the incidence of CWD in the wild elk was less than 1% in 1998, making it unlikely that many calves had been infected from their dams prior to capture in 1986 (Miller, Wild & Williams, 1998). It was considered most likely that “maternal transmission” should be seen as special

cases of lateral transmission, occurring between infected females and their offspring (Miller, Wild & Williams, 1998).

171. Lateral (rather than maternal) transmission was further indicated to be the main transmission route for CWD in mule deer by showing that the overall incidence of CWD in two cohorts of mule deer at a research centre was similar (Fisher's exact test, P approximately 1.0), as were mean intervals to death, despite the fact that one cohort might have experienced maternal transmission (since all except one dam of this cohort eventually developed CWD) while individuals in the other were presumed not to be infected, as the fawns came from an enclosed population in which CWD has not been detected despite extensive sampling (0 positive results from 198 samples; 95% confidence interval for prevalence 0-1.9%). Additionally, the cohort-specific incidence increased for later cohorts within the herd and the mean age at death showed a sharp decrease; these findings also indicated horizontal transmission (Miller & Williams, 2003). Epidemiological investigations of an outbreak in the 1986 cohort of female elk investigated in the Foothills Wildlife Research Facility, Fort Collins, Colorado, indicated that periparturient spread is not required for transmission of CWD (Miller, Wild & Williams, 1998).

172. A comparison of the dynamics of epidemics in white-tailed deer and mule deer cohorts at a research facility indicated that the dynamics in the two species were similar. In white-tailed deer surviving more than 15 months in the facility, clinical CWD developed in nine of 11 animals (82%); age at death or euthanasia due to terminal CWD was 49 to 76 months (mean 59.6 months, SE 3.9 months). In hand-reared mule deer four of six animals developed clinical CWD, dying at 64 to 86 months (mean 72 months, SE 5 months) and in the cohort of dam-raised mule deer CWD developed in four of six animals, with death at 31-58 months (mean 41.3 months, SE 6.1 months). Differences in mean age at death due to CWD were considered probably to be related to differences in either the timing or the intensity of exposure to CWD (Miller & Wild, 2004).

173. It is likely that the level of infection in captive herds of cervids will depend on the duration of exposure to the CWD agent (either direct or indirect) and the amount of the agent in the local environment; this should lead to high levels of infection in animals maintained in highly contaminated areas for long periods (Kahn *et al.*, 2004). In infected herds in Saskatchewan only 4 of 42 herds (9.5%) had a high (14% to 33%) prevalence of CWD, while 65% of herds had a prevalence of less than 5% (Salman, 2003; Kahn *et al.*, 2004). Based on this data, and the lack of clinical signs in 91% of the CWD-positive cervids in these herds, it is assumed that in most of the herds CWD was detected soon after it has been introduced (Kahn *et al.*, 2004). Indirect transmission, presumably via a contaminated environment, may best explain observed dynamics of epidemics in captive deer (Wild & Miller, 2004).

174. In research facilities, epidemics of CWD have affected very high percentages of the populations and tended to drive the population to extinction (Williams & Young, 1992; Miller *et al.*, 2000).

Epidemiology and spread in free-ranging cervids

175. Spread of CWD between geographical areas is probably due to movement of live animals (Samuel *et al.*, 2003). This may include natural movements of infected animals, and translocation of both captive and free-ranging cervids (Miller *et al.*, 2000; Gross & Miller, 2001; Williams *et al.*, 2002; Bollinger *et al.*, 2004).

176. In at least three areas (west-central Saskatchewan, northwest Nebraska and southwest South Dakota) it is possible that an elk farm infected with CWD may have acted as a source

of infection for the local populations of free-ranging cervids (Williams *et al.*, 2002; Miller & Williams, 2004). In Canada, while spread from farmed deer was almost certainly the source of infection for one focus in free-living deer, and was probably the source for another focus, the disease has since spread geographically in these populations and there are no obvious natural barriers which would prevent further spread (Bollinger, 2004).

177. Dispersing deer probably act as a method of spread of CWD within a geographical area (Williams *et al.*, 2002; Bartelt, Pardee & Thield, 2003; Bollinger, 2004). Data on prevalences in Colorado and Wyoming are consistent with known movements of mule deer in these regions (Miller *et al.*, 2000). Looking at the situation in Wisconsin and Illinois, it is known that in white-tailed deer, while female fawns commonly remain within their natal social group and in the same geographical area as their mother, male fawns usually disperse at about 12 to 18 months old; average dispersal distances in Wisconsin are only a few miles, but longer movements, greater than 30 miles, have been recorded occasionally for deer in the Midwest (Bartelt, Pardee & Thield, 2003). A study in Minnesota recorded dispersal of white-tailed deer as far as 168 km, although most yearlings moved 38 km or less (Nelson, 1993).

178. Based on a study comparing CWD epidemiology in captive white-tailed and mule deer (see paragraph 172), it was considered that epidemics in sympatric deer of these two species in shared habitats in the western North American ranges might be similar to one another (Miller & Wild, 2004).

179. The social behaviour of deer, particularly “yarding” behaviour in winter, may concentrate populations and increase the chance for lateral transmission to occur, whether directly or indirectly (Bartelt, Pardee & Thield, 2003). Additionally, lateral transmission may be enhanced artificially when deer congregate at feeding or baiting sites. (Williams, Miller & Thorn, 2002; Bartelt, Pardee & Thield, 2003; Van Deelen, 2003; Miller & Williams, 2003; Bollinger *et al.*, 2004). Deer feeding on frozen food piles use heat from their own nose and mouth to thaw the food and leave both saliva and nasal droppings behind (Van Deelen, 2003; Bartelt, Pardee & Thield, 2003). Transmission may occur faster in the eastern USA areas where the population density of white-tailed deer is much greater (typically by an order of magnitude) than in the western deer and elk populations where CWD has been known to be endemic for decades (Joly *et al.*, 2003). Transmission probably occurs between sympatric populations of the different cervid species (Samuel *et al.*, 2003).

180. It has been demonstrated experimentally that CWD can be transmitted via carcasses and via environments contaminated by excreta. Obviously, excreta and carcasses remaining naturally on native ranges will be available to act as a source of infection for free-ranging animals (Williams & Miller, 2003; Miller *et al.*, 2004). Cervids such as mule deer, living in established home ranges, will have more frequent encounters with such sources of contamination than they would if their movements were random (Miller *et al.*, 2004).

181. Epidemiological studies involving mathematical modelling have suggested that CWD in free-living cervids is likely to result in population declines and even extinction (Miller *et al.*, 2000; Gross & Miller, 2001). An individual model was used, with deer being susceptible, latent (infected but not infectious), infected (and shedding) or dead. The model “*assumed that each infectious individual produced an estimable number of infectious contacts per unit time and that all individuals in the host population had an equal probability of contracting an infectious dose.*” The formula used produced transmission rates that were “largely independent” of overall population density. This was considered appropriate for mule deer and most other cervids “*that tend to aggregate or use habitats in relation to quality*” (Gross & Miller, 2001). It was noted that transmission via environmental contamination was not

represented in the models (Miller *et al.*, 2000). A critique of the models suggested that, particularly given the probable combination of direct and indirect transmission a frequency-dependent model might not fully reflect actual factors affecting transmission. It was suggested that, particularly because of congregations of cervids on winter ranges, where exudates from an infected individual might potentially contact a larger number of animals if more animals are congregating in or moving through a given area, there may be a density-dependent effect on transmission (Schauber & Woolf, 2003). Clearly further work is required on modelling of CWD epidemiology in free-living cervids.

8) SUSCEPTIBILITY WITHIN THE KNOWN NATURAL HOSTS

182. Data from studies on elk and white-tailed deer suggest that breeding for genetic resistance to CWD is not a practical option (Johnson *et al.*, 2003).

Genetics in *Cervus elaphus nelsoni*

183. Early coding of the PrP gene in *Cervus elaphus canadensis* (wapiti) found leucine (Leu) at codon 129 (Schätzl *et al.*, 1997) (this is cervid codon 132 – see O'Rourke *et al.*, 1999). Later work showed that Rocky Mountain elk have a polymorphism (Met/Leu) at this codon (cervid codon 132) (O'Rourke *et al.*, 1998a; O'Rourke *et al.*, 1999).

184. In a study looking at the genotypes of both free-ranging and captive Rocky Mountain elk with CWD, Met/Met homozygotes were over-represented among CWD-positive animals compared with control groups not affected by CWD, and no Leu/Leu animals were CWD-positive. However only a few Leu/Leu animals were tested so it could not be stated that this definitely showed resistance of the Leu/Leu genotype individuals. It was noted that the commonest CWD-positive genotype (Met/Met) was also the commonest genotype in the population (75.1 % of the wild population) while Leu/Leu homozygotes made up only 1.1% of the population (O'Rourke *et al.*, 1999). A later study on 10,269 captive elk found that 96.7% of CWD-positive individuals were M/M homozygotes with one CWD-positive L/L homozygote and seven L/M heterozygotes (Spraker *et al.*, 2004). When scrapie was inoculated intracerebrally into six elk, the last of the elk to develop spongiform encephalopathy was L/M heterozygous at codon 132; the other two individuals which became infected were both homozygous M/M (Hamir *et al.*, 2004b). It has been suggested that experimental pathogenesis and oral challenge trials in elk of all three genotypes will be required to determine whether there are any differences in disease progression between the genotypes, such as a prolonged incubation period in heterozygotes (Spraker *et al.*, 2004).

185. Recent data suggests that genotype does affect incubation time in elk; incubation is shorter in individuals homozygous for methionine at codon 132 (Williams & Miller, 2004).

186. An unexpressed pseudogene, as detected in the two *Odocoileus* spp. deer (see below), has not been detected in *Cervus elaphus nelsoni* (O'Rourke, 2004; Spraker *et al.*, 2004).

Genetics in *Odocoileus hemionus*

187. Initial comparison of coding of the PrP gene in healthy Rocky Mountain elk (*Cervus elaphus nelsoni*) and mule deer (*Odocoileus hemionus*) revealed 99.6% similarity, with glutamine (gln, Q) at codon 226 in the mule deer whereas glutamic acid (glu, E) was found in this position in the elk; there was also a polymorphism at codon 138 in the mule deer, with either serine (Ser, S) or asparagine (Asn, N) at this position (Cervenáková *et al.*, 1997). Raymond *et al.* (2000) confirmed the difference at codon 226 between *Cervus elaphus nelsoni* and *Odocoileus* spp. deer and also detected the polymorphism S/N at codon 138 in the deer. Work by O'Rourke *et al* showed three alleles, two alleles coding S and N respectively at

codon 138 for alleles designated 138S2 and 138N1, with the third allele, designated 1238S1, differing from 138S2 by a silent mutation (O'Rourke *et al.*, 1998a).

188. Later studies (Brayton *et al.*, 2004) on 145 samples found that 144 of the 145 were heterozygous at codon 138: serine [S] / asparagine [N] (agc change to aac) as well as non-coding polymorphisms at codon 139 (agg/aga) and codon 156 (aat/aac). Two to four PRNP alleles were detected per individual, with either one or two alleles encoding 138 S in every individual and one or two alleles always coding 138 N. It was determined that a gene duplication event had occurred: in addition to a full length functional gene there was a processed pseudogene, which is not translated. The single deer that typed homozygous for 138S was found to lack the pseudogene. Within the functional gene three alleles were detected; coding changes were found only at codon 20 (aspartic acid [D] or glycine [G] and at codon 225 serine [S] or phenylalanine [F]; all the functional gene alleles coded S at codon 138. There were also non-coding changes at codons 131, 146, 156, 202 and 206. The functional gene coded for S at codon 138 while the pseudogene coded for N at this location. Out of the six possible peptide combinations for the functional gene sequences, four combinations were found in the 47 CWD-positive mule deer tested: D₂₀S₂₂₅-D₂₀S₂₂₅, D₂₀S₂₂₅-D₂₀F₂₂₅, D₂₀S₂₂₅-G₂₀S₂₂₅ and G₂₀S₂₂₅-G₂₀S₂₂₅; 34 (72.3%) CWD-positive animals were homozygous for D₂₀S₂₂₅, one (2.1%) was homozygous for G₂₀S₂₂₅ while eight (17.0%) were heterozygous at codon 20 and four (8.5%) were heterozygous at codon 225. None of the 47 CWD-positive deer were found to be D₂₀F₂₂₅-D₂₀F₂₂₅ or D₂₀F₂₂₅-G₂₀S₂₂₅, but since the allele D₂₀F₂₂₅ was rare much larger sample sizes will be required to determine whether this allele affects susceptibility (Brayton *et al.*, 2004).

189. While no genotype is known to confer resistance to CWD infection, genotype does affect incubation times for CWD in this species; the incubation time is shorter for mule deer which are homozygous for serine at codon 225 compared to individuals heterozygous S/F at this codon (no data is yet available for incubation time in the rare F/F252 homozygote) (Williams & Miller, 2004).

Genetics in *Odocoileus virginianus*

190. The first polymorphisms detected in the PRPN of *Odocoileus virginianus* – white-tailed deer were at codon 96 glycine [G]/serine [S] and codon 138 serine [S]/asparagine [N]; in addition it was noted that, as with *Odocoileus hemionus*, this species differed from *Cervus elaphus nelsoni* by coding for Q rather than E at codon 226 (Raymond *et al.*, 2000). Johnson *et al.* (2003) detected in addition a polymorphism at codon 95: glutamine [Q] to histidine [H]. The QGS allele (glutamine at codon 95, glycine at codon 96 and serine at 138) has been referred to as the “wild-type” allele (Raymond *et al.*, 2000, Johnson *et al.*, 2003). CWD-positive QGS/QGS, QGS/QGN and QGS/QSS deer were all detected in free-ranging white-tailed deer in Wisconsin. It was noted that, while no significant differences were found, trends indicated that the QSS allele was underrepresented within CWD-positive individuals (Johnson *et al.*, 2003). This was not expected given that data from cell-free conversion studies had indicated little or no molecular barrier to conversion of the QSS allele to the PrP-res form (Raymond *et al.*, 2000; Johnson *et al.* (2003). Note: this work was carried out before the presence of the PRPN pseudogene was recognised: see below, paragraph 191.

191. More recent work, involving testing of 133 individuals from an enclosure in western Nebraska, USA, in which 50% of the deer were CWD-positive revealed a functional gene and a pseudogene (O'Rourke *et al.*, 2004). Three polymorphisms encoding amino acid substitutions within the functional gene: glutamine [Q] or histidine [H] at codon 95, glycine [G] or serine [S] at codon 96 and alanine [A] or glycine [G] at 116; all alleles of the functional gene encoded serine [S] at codon 138. There were four functional alleles: QGAS,

QSAS, QGGS and HGAS with allelic frequencies 0.50, 0.36, 0.13 and 0.011 respectively. In 26% of the deer a processed pseudogene was identified also, with two alleles, showing five or six copies of the octapeptide repeat and both of which encoded asparagine [N] at codon 138. The pseudogene was found in individuals of all the major PRNP genotypes and, as expected for a non-functional gene, did not correlate with CWD status. Of the ten potential diploid genotypes (for the functional gene), nine were detected in the herd; no homozygotes of the rarest allele, HGAS, were present. All five of the commonest diploid genotypes in the herd were found in white-tailed deer with CWD. While no QGGS homozygotes or HGAS/QGAS individuals were found to be CWD-positive there were few deer with these genotypes within the herd (two of each), so conclusions cannot be drawn. Within the five major diploid genotypes the frequencies of CWD-positives differed (chi-squared 12.2, 4 df, $p=0.016$) and in particular deer haploid or diploid for QGAS were overrepresented within the CWD-affected animals, while those carrying one or two QSAS alleles were underrepresented among the CWD-positive animals. However there was no evidence of resistance to CWD in deer with any of the major alleles in this study (O'Rourke *et al.*, 2004).

192. No additional markers associated with resistance to CWD have been detected by microsatellite analysis of DNA samples from a herd of white-tailed deer which is heavily infected with the disease (O'Rourke, 2004).

193. Differences in the PRPN gene between species, and polymorphisms within species for the known natural hosts of CWD are provided in Table 2. See Table 3 for a comparison of PRNP sequences from various North American and European cervid species.

Age specific susceptibility?

194. It is probable, based on age-class specific prevalence data from wild cervids and epidemiological evidence from captive cervids in affected research centres, that both adults and fawns may become infected with CWD (Miller, Wild & Williams, 1998; Miller *et al.*, 2000).

195. Most cases in the research facilities in Colorado and Wyoming where the disease was first investigated occurred in animals of three to four years of age (Williams & Young, 1992). Early-stage disease with PrP^{CWD} found only in the lymphoid system, not in the CNS, has been identified in adult white-tailed deer of estimated age five years or older, suggesting either infection as adults or extremely prolonged incubation (O'Rourke *et al.*, 2004).

196. In Rocky Mountain elk, clinically affected individuals have been diagnosed from about 17 months old to 15 years old while infection has been diagnosed by immunohistochemistry (IHC) in individuals from one year old upwards. Within free-living elk submitted from northcentral Colorado, March 1981- June 1995 due to suspicious clinical signs and diagnosed with CWD the youngest affected animal was 1.8 years old and the oldest 15 years old (Spraker *et al.*, 1997). In 4/23 female elk (17%) from a cohort hand-reared at a wildlife research facility in Colorado in 1986 the age at onset of clinical signs was about 2.9 to 8.1 years (Miller, Wild & Williams, 1998). Within farmed elk, in a herd presumed to be recently infected one individual was older than 15 years (Williams, Kirkwood & Miller, 2001). The earliest age of diagnosis in an elk from a privately owned game farm in South Dakota was 17 months, while other affected animals from that herd were at least two years old (O'Rourke *et al.*, 1999). In 46 clinically normal elk on an infected elk farm in Saskatchewan, Canada, shown to be CWD-positive by IHC, the age range was one to 13 years (Balachandran *et al.*, 2002). In another captive herd, the presence of PrP^{res} in the brain was confirmed in ten of 17 animals, including four animals only two years old and four animals of three years old. Clinical signs were present in two animals three and five years old, with

histological lesions consistent with CWD in one of the two-year-old animals and mild lesions suggestive of CWD in another three-year-old animal (Peters *et al.*, 2000). Testing of 10 269 elk from farmed elk herds in the USA and Canada in which at least one animals had been found to be CWD-positive detected CWD-positive animals of all ages from about 18 months to more than 12 years old (Spraker *et al.*, 2004).

197. In mule deer, deer diagnosed with CWD in captive research facilities in Colorado and Wyoming were all adults of 2.8 to 4.0 years old (Williams & Young, 1982). In free-living individuals from northcentral Colorado diagnosed with CWD between March 1981 and June 1995, the estimated age range was 2.5 to 7.5 years old (Spraker *et al.*, 1997). A study of naturally-infected individuals, based on immunohistochemical staining and histological lesions, suggested that the progression of disease may be more rapid for individuals infected early in life (Spraker *et al.*, 2002b).

198. In *Odocoileus virginianus* – white tailed deer, out of 179 white-tailed deer which had become enclosed by an elk farm fence, in Sioux County, northwestern Nebraska, four fawns only eight months old were among the 50% of CWD-positive animals; these fawns were not showing any clinical signs of CWD (Davidson, 2002). In a study of 500 deer of one year old or older in southern Wisconsin in 2002, within an area radius approximately 18 km, from 476 useable samples there was a trend towards an increase in prevalence with age; only 32 samples were from animals more than five years old, which reduced the study's ability to confirm whether there was a statistically significant increase in prevalence with age (Joly *et al.*, 2003).

199. A functioning immune system appears to be required for transmission of the TSE agent from peripheral sites (Dickinson & Outram, 1979; Mabbott & Bruce, 2001). It has been suggested that young animals, with a more active immune system, may be more susceptible to peripheral infection with TSEs than older animals; experimental verification would require a crossover design with young and older animals inoculated peripherally and intracerebrally (Heisey & Joly, 2004).

Effect of sex on susceptibility?

200. Male, female and castrated animals have all been affected (Williams *et al.*, 1990). A study of free-living mule deer, elk and white-tailed deer in Colorado and Wyoming indicated no obvious sex-related difference in prevalence (Miller *et al.*, 2000). However in mule deer in Colorado and Wyoming data to the end of 2003 showed a higher prevalence in males than females in the age class three years and older (T. R. Kreeger, pers. comm.). Increased prevalence in males over females has also been noted in white-tailed deer in Wisconsin (Langenberg, 2004). Field observations in Canada indicate that within elk, males and females are equally susceptible (Kahn *et al.*, 2004).

201. In a study of 500 white-tailed deer of one year old or older in southern Wisconsin in 2002, within an area radius approximately 18 km, from 476 useable samples, prevalence was not found to vary with sex: 3.4% of 87 males (95% CI 0.1% - 9.7%) and 3.1% of 386 females (95% CI 1.6% - 5.3%) were CWD-positive (Joly *et al.*, 2003). Further studies in Wisconsin deer have found prevalence in adult males to be higher than prevalence in adult females. It was considered that males might have a higher exposure than females to infection due to larger home ranges increasing their likelihood of contacting CWD, females passing disease to males during the mating season (when males visit many females), or due to the formation of bachelor groups of males in which the males have closer physical contact with one another, which may make spread of disease easier (Wisconsin Department of Natural Resources, 2004a).

9) ANIMAL HEALTH CONCERNS

202. One concern regarding CWD is the possibility that it may be transmitted to other species. Interspecies barriers to transmission of TSEs are not fully understood and probably involve the nature of interactions between disease-associated prions and cells at sites of entry, and efficiency of translocation of prions to the CNS from peripheral sites of entry, as well as the “species barrier” or molecular barrier to conversion of host prions by the infecting agent (Belay *et al.*, 2004; Gould *et al.*, 2003; Raymond *et al.*, 2000). In addition to concerns regarding the disease in other species *per se*, there is a concern that if CWD was transmitted to domestic livestock such as sheep and cattle then this would increase the risk of transmission to humans, since there would be a potential increase in extent and frequency of exposure of humans to the CWD agent and, furthermore, passage through domestic livestock might alter the infectivity of the agent, possibly increasing its infectivity to humans (Belay *et al.*, 2004).

Potential risk to other Cervidae

203. It is assumed that other subspecies of the cervid species known to be natural hosts (i.e. subspecies of *Cervus elaphus*, *Odocoileus virginianus* and *Odocoileus hemionus*) would also be susceptible. This includes the European red deer *Cervus elaphus elaphus* (Williams *et al.*, 2002). See paragraph 60 for a note on taxonomy and species limits.

204. The risks of transmission to other deer species are not yet known. As indicated above (paragraph 78) oral transmission has been successful in one moose but it is not possible to draw general conclusions from this single case; no data is yet available regarding the dose of inoculum or the number of animals inoculated. CWD has never been diagnosed in any free-ranging moose (Colorado Division of Wildlife, 2004c).

205. An important factor in interspecific transmission of TSEs appears to be the molecular compatibility (degree of homology) between the infecting PrP and the endogenous cellular PrP of the new host; this is commonly known as the “species barrier” (Raymond *et al.*, 2000; Belay *et al.*, 2004). The PrP sequences of various cervids found in Europe have been compared with the PrP sequences of the known natural hosts of CWD. A study of the prion protein of cervids from Sweden indicates that European moose (*Alces alces alces*) roe deer (*Capreolus capreolus*) and semi-domestic reindeer (*Rangifer tarandus tarandus*) of Swedish origin carry the allele Q95G96A116S138 that is over represented in CWD affected mule deer and white-tailed deer in North America. Fallow deer (*Dama dama*) in this study were homozygous for the Q95G96A116N138 allele. It was noted that silent nucleotide differences were detected between samples, showing that the results were not due to cross-contamination during the PCR (Simonsson, Klingeborn & Linne, 2004). O’Rourke *et al.* noted that the pseudogene found in the *Odocoileus* spp. deer had not been detected in several other species: “No evidence of the pseudogene was found in small populations of New World moose (*Alces alces*), holarctic reindeer, Old World Rocky Mountain elk (*C. elaphus*) or captive Asian fallow deer (unpublished data)” (O’Rourke *et al.*, 2004). A comparison of PRNP sequence variations from some North American and European cervid species is provided in Table 3.

Surveillance studies

206. Surveillance studies on deer in Europe have not yet detected any cases of TSE (A.M. Barlow, pers. comm., 2004; Sigurdarson, 2004; Schettler *et al.*, 2004; Schwaiger *et al.*, 2004; Sieber *et al.*, 2004).

Reindeer (caribou) – *Rangifer tarandus*

207. No TSE has been detected to date in *Rangifer tarandus* – reindeer (caribou) North America or Europe. During 2000, 100 caribou from northern Quebec, Canada, were tested for CWD using histology and immunohistochemistry (primary antibody F99/97.6.1, which has known reactivity against the abnormal PrP of CWD-affected mule deer and Rock Mountain elk, scrapie-affected sheep and BSE-affected cattle); no cases of TSE were found, although it was acknowledged that this level of testing of a herd numbering hundreds of thousands would only be expected to detect any cases if the prevalence in the herd was 6.6% or more (assuming that testing by immunohistochemistry had a 99% sensitivity and specificity) (Lapointe *et al.*, 2002). Formalin fixed brain samples from 196 *Rangifer tarandus* - reindeer from Norway have been examined histologically; no histological signs of any TSE have been detected. Bio-Rad ELISA of frozen brain samples is planned (Sigurdarson, 2004).

Common European deer species – red deer *Cervus elaphus elaphus*, roe deer *Capreolus capreolus* and fallow deer *Dama dama*

208. In Bavaria, brain samples of 654 roe deer *Capreolus capreolus* and 189 red deer *Cervus elaphus elaphus* were tested for TSE using the BioRad ELISA (brain samples of positive controls, known CWD-positive *Cervus elaphus nelsoni* were positive using this test); 10% of samples were also tested by immunohistochemistry using monoclonal antibody L42. None of the brains were positive for TSE using either test. The results gave a 95% certainty for incidence of TSE at under 0.5% for roe deer and under 1.4% for red deer (Schwaiger *et al.*, 2004). In Germany as a whole a study is underway which aims to have a 95% probability of detecting at least one positive case of a TSE in cervids if TSE is present at a prevalence of 0.5% or higher; this will require testing of 7,000 free-living cervids (3,000 roe deer, 2,000 red deer and 2,000 fallow deer) as well as 1,000 captive cervids. Sampling is based on hunter harvested deer as well as a targeted survey. Animals of 18 months old or older are included in the study and both obex and retropharyngeal lymph nodes are sampled and tested using the BioRad ELISA and in some cases also using immunohistochemistry. Samples taken from August 2000 onwards from more than 4,000 cervids in Germany (roe deer, red deer and *Dama dama* - fallow deer) have all been negative for TSE. (Schettler *et al.*, 2004).

209. In the UK, samples collected during 2000-2003, were tested from 304 cervids (189 roe deer (*Capreolus capreolus*), 22 red deer (*Cervus elaphus elaphus*), 66 fallow deer (*Dama dama*), 13 muntjac (*Muntiacus muntjak* (*Muntiacus reevesi* – see Chapman, 1991)) and 14 of unknown species), which had either been harvested or picked up as road-kills in the West of England. Obex, mesenteric lymph node, medial retropharyngeal lymph node, Payer's patch (distal ileum) and spleen were collected, as available. Tissues were fixed and tested by immunohistochemistry using monoclonal antibody F89/160.1.5 (with monoclonal antibody F99/97.6.1 for confirmation of equivocal staining). This testing has failed to detect any prion disease in the deer. Some staining of intestinal tissues occurred with F89/160.1.5, which may have been detection of PrP^C. F99 would be the monoclonal antibody of choice for further work (A.M. Barlow, VLA Wildlife Group, pers. comm. citing VLA unpublished report, 2004). There is an ongoing limited survey of wild deer and more active surveillance of culled "park" deer (A.M. Barlow, pers. comm., 2004).

210. In Switzerland, surveillance for CWD in farmed cervids has started, with a programme aiming to look at 200 adult cervids of two years old or older. Most (85%) of farmed deer in Switzerland are fallow deer, with smaller numbers of red deer (10%) and sika deer (*Cervus nippon*) (5%). During 2003, 72 adult deer (mainly fallow deer, a few red, sika and roe deer) were examined by histopathology of the brain, tonsils, lymph node and third eyelid and no signs of spongiform encephalopathy were detected; IHC results are pending (Sieber *et al.*, 2004).

Potential risk to domestic cattle & sheep

211. To date, no transmission of CWD has been reported in domestic species living in CWD endemic areas or in research facilities with CWD. Monitoring is ongoing (Gould *et al.*, 2003; Williams & Miller 2002; Belay *et al.*, 2004). It has been possible to infect domestic cattle, goats and sheep by intracerebral inoculation, although not in all inoculated individuals (Hamir *et al.*, 2003a; Hamir *et al.*, 2004a); see paragraphs 66-67. Data from intracerebral inoculation experiments show that diagnostic methods currently in use for BSE surveillance would detect the CWD agent in cattle and sheep if it were present (Hamir *et al.*, 2003a).

212. Data from *in vitro* experiments suggests that there may be a considerable “species barrier” limiting transmission of CWD from cervids to domestic cattle and, to a lesser extent, to domestic sheep. In a cell-free conversion system, PrP^{CWD} from elk, mule deer or white-tailed deer showed 5-to-12-fold lower conversion efficiency of bovine PrP-sen than for inter-cervid conversion reactions; conversion efficiency of ovine PrP-sen (ovine PrP-AQ) was also less than half as efficient as for homologous cervid reactions (Raymond *et al.*, 2000).

213. There is a theoretical risk than CWD could be transmitted to cattle via incorporation of infected tissue from Cervidae into meat and bone meal. The risk for this occurring in the USA was considered [in 1992] to be small “*because CWD is believed to be rare and localized, and the proportion of harvested Cervidae whose offal is rendered is probably small*” [in the USA] (Saunders, 1994). The feeding of ruminant-derived protein to ruminants has been banned in Canada and the USA since 1997 (Kahn *et al.*, 2004). The US Food and Drug Administration (FDA) has, since November 2002, banned the use of “*material from Chronic Wasting Disease (CWD)-positive animals, or animals at high risk for CWD, to be used as an ingredient in feed for any animal species.*” Animals considered to be at high risk for CWD were stated to include animals from CWD-positive captive herds, free-ranging animals from the CWD-endemic area in Colorado and Wyoming, deer from the CWD eradication zone in Wisconsin and also “*deer from any areas designated around any new foci of CWD infection that might be identified through surveillance or hunter harvest testing*” (FDA, 2002). Such policies should minimise the potential oral exposure of domestic ruminants to CWD-agent in feed.

Potential risk to other species

214. Since BSE appears to have been transmitted orally to various Felidae (Kirkwood *et al.*, 1995; Bourne, 2004b), the possibility of CWD being transmitted to carnivores must be considered.

215. Experimentally, CWD has been successfully transmitted by intracerebral inoculation to domestic ferrets (*Mustela putorius fero*) and to American mink (*Mustela vison*), but not to common raccoons (*Procyon lotor*) (Williams, Young & Marsh 1982; Williams & Young, 1992; Williams *et al.*, 1992; Hamir *et al.*, 2003c; Sigurdson *et al.*, 2003). Since raccoons are highly adaptable carnivores which may include carrion in their diet (Hamir *et al.*, 2003c), the lack of success in transmission of CWD to raccoons even by intracerebral inoculation is encouraging.

216. There is no published data on transmission or attempted transmission of CWD to felids or canids.

10) HUMAN HEALTH CONCERNS

217. To date there are no known cases of human prion disease attributable to CWD transmitted to humans (Belay *et al.*, 2004). While limited epidemiological investigations to date have not shown any links between CWD and humans with spongiform encephalopathies

this data must be considered along with a caveat: “because CWD is a relatively new TSE, it is unlikely that enough people have consumed enough CWD-affected cervids to result in a clinically or pathologically recognizable disease attributable to CWD, especially considering the very long incubation periods characteristic of TSE diseases.” (Race *et al.*, 2002)

Epidemiological investigations

218. Epidemiological investigations have failed to show any links between cases of prion disease in unusually young people or in hunters in the USA and CWD (CDC, 2003). Two major epidemiological investigations have been carried out, one on cases of CJD in unusually young individuals in the USA, the second on a group of men from Wisconsin who developed neurological diseases.

219. The first study (Belay *et al.*, 2001) focused on three individuals, two 28 years of age and the third 30 years old, diagnosed with CJD in the USA between 1st January 1997 and 31st May 2000, and without any established risk factors for CJD (family history, receipt of human growth hormone, receipt of grafts of dura mater or cornea, or previous neurological surgery) and concluded that there was no strong evidence for a causal link with CWD. None of the individuals had travelled to Europe (therefore a link with BSE was unlikely). Two of the individuals were hunters who regularly consumed game meat while the third (case 1) had, as a young child, regularly consumed venison from animals hunted by family members and on two occasions from a family friend. Two of the individuals (cases 1 and 2) had undergone tonsillar surgery as children; the third had never received any surgical treatment. One individual (case 1) had eaten venison mainly hunted in Maine, occasionally hunted in New Jersey, and, on two occasions at about six years old, elk meat which had probably been harvested in Wyoming. The second person (case 2) had hunted cervids mainly in Utah, but had harvested an elk in southwestern Wyoming on one occasion (less than three years before onset of clinical signs) and had hunted in British Columbia on one occasion nine years before onset of illness. The third person (case 3) had hunted close to home and never in Colorado or Wyoming although the plant where he took his carcasses for processing did also process some elk from Colorado each year. The clinical signs, duration of illness and histopathological findings for the three individuals showed no obvious similarities to one another. One individual was methionine/methionine homozygous at codon 129 of the PRNP (case 1), one was homozygous for valine at this gene (case 2) and the third (case 3) was heterozygous methionine/valine. Immunohistochemistry revealed strong staining with a “synaptic” pattern in the first individual and weak staining with a “synaptic” pattern in the second case; in case 3, based on a brain biopsy sample obtained at an early point in the illness, staining was questionable and possibly showed a synaptic pattern. Cases 2 and 3 showed a “Type 1” immunoblot pattern, this test had not been carried out for case 1. It was noted that none of these three individuals had a definite history of consumption of venison from the geographical areas in which CWD was known to be endemic in Colorado and Wyoming, and no CWD had been identified in 299 deer and sampled from the area in which most of the venison consumed by patient 1 had originated, nor in 404 deer and 196 elk sampled from the area in which most of the venison consumed by patient 2 had originated, nor in 138 deer samples from the area in which most of the venison consumed by patient 1 had originated. Additionally, there was no homogeneity in phenotypic expression of the disease and all three possible options for coding at codon 129 of the PRNP gene were represented. Since a survey had indicated that approximately 40% of blood donors in the USA consumed venison from wild cervids, it was considered most likely that coincidence explained why three of the four young (30 years old or younger) individuals with sporadic CJD reported in the USA after March 1996 had consumed such meat (Belay *et al.*, 2001).

220. The second major epidemiological investigation centred around three men from Wisconsin and Minnesota who had died from degenerative neurological illnesses and who had participated in “wild game feasts” in northern Wisconsin. Full investigation including examination of fixed brain tissue confirmed CJD in only one of the three individuals. Wild game eaten during the feasts was harvested mainly in Wisconsin but also in areas of Colorado, Wyoming and Montana; CWD was not known to be endemic in the areas where the game was hunted at the time that the game was harvested. Further investigations of other possible attendees of the feasts revealed 34 participants, all male, of whom a total of seven were deceased, including the three individuals in the initial investigation. Causes of death in the other four deceased individuals were not attributed to nor associated with any degenerative neurological disorder and no signs or symptoms associated with a degenerative neurological disorder were noted for any of the remaining living participants of the feasts. It was noted that only one case of CJD had occurred among known participants at the feasts, that this case was consistent with the commonest form of sporadic CJD, that this individual had only participated in one feast and that it was unlikely that he had consumed CWD-infected venison at the feasts “*because venison and other game from outside Wisconsin that was served at these feasts did not originate from known CWD-endemic areas.*” Limitations of the investigations were noted to include reliance on recall of events from up to 25 years previously and the fact that not all participants in the feasts could be contacted and interviewed. However, those who were interviewed agreed in their recall of events (CDC, 2003).

221. It is important to recognise that the limited epidemiological investigations that have been carried out are not able to rule out the possibility that CWD might play a role in causing illness in humans (CDC, 2003).

222. Three further cases of prion disease in young humans in the USA have been investigated for possible links to CWD (Belay *et al.*, 2004). The first case was a 25-year-old man who died in 2001 after about 22 months of illness. Gerstmann-Sträussler-Scheinker syndrome (GSS) was diagnosed by analysis of the prion gene, with a P102L mutation together with valine at codon 129 in the mutant allele. It was noted that the disease had occurred at an unusually young age, even for GSS, and the possibility that exposure to CWD-infected venison contributed to early onset of the disease could not be ruled out; the patient’s grandfather had regularly hunted in southeastern Wyoming, around the known CWD-endemic area, and had given venison to the patient’s family. Two other cases of prion disease occurred in individuals of 26 and 28 years of age, from adjacent counties, and with onset of illness only months apart, therefore an environmental source of infection was investigated. However, these two individuals were finally diagnosed with different prion diseases: sporadic CJD in one case and GSS in the other, indicating that a common cause was unlikely. In the first case CJD was confirmed from autopsy samples (by histopathology, immunohistochemistry and immunoblotting); the individual had no history of hunting nor of regular consumption of venison, and although he may have eaten venison originating from the Upper Peninsula of Michigan while at college CWD has never been detected in deer from Michigan. Phenotypically this individual fit the “MM2 sporadic CJD” phenotype described by Parchi *et al.* (1999). In the other case *post mortem* immunohistochemistry revealed prion deposition which was consistent with GSS and a GSS P102L mutation was detected in a blood sample from one parent (appropriate samples were not available from the affected patient); this individual may possibly have eaten venison from Michigan on one occasion at about two years of age (Belay *et al.*, 2004).

223. A further three cases of CJD in individuals of 54 to 66 years old who were deer and elk hunters (two individuals) or ate wild-harvested venison (one individual) have been

investigated. There was no evidence that any of these individuals had hunted in known CWD-endemic areas; information available indicated hunting or eating venison from Washington State and Pennsylvania. Two individuals were V/V at codon 129 the third was M/M; they were considered to fit known subtypes of sporadic CJD (MM1, VV1 and VV2 subtypes as described by Parchi *et al.* (1999)). Further investigations were also made on the only two nonfamilial cases of CJD in individuals with a history of eating venison from the known CWD-endemic areas. One was reported to have eaten venison from two deer harvested in an area with endemic CWD, but both deer had been tested and not found to be CWD-positive; the patient's illness was consistent with the CJD subtype MM1. The other individual grew up in a CWD-endemic area and ate locally-harvested venison; her disease fit the MM1 CJD phenotype and no atypical neurological features were noted (Belay *et al.*, 2004).

224. Additional epidemiological notes are that the incidence and age distribution of CJD in Colorado and Wyoming, where CWD is thought to have been endemic for decades, are similar to those found in other areas of the USA. In Wyoming, seven cases of CJD have been reported between 1979 and 2000 with an average annual age-adjusted CJD death rate of 0.8 per million and no cases reported in humans less than 55 years old. In Colorado in the same period 67 cases of CJD have been reported, with an average annual age-adjusted CJD death rate of 1.2 per million (Belay *et al.*, 2004).

225. In summary, there is no evidence of an increase in incidence of CJD in Colorado and Wyoming, nor have epidemiological investigations carried out so far found any evidence of a link between CWD and cases of CJD in persons in the USA (Belay *et al.*, 2001; CDC, 2003; Belay *et al.*, 2004).

Laboratory studies

226. There is evidence from an *in vitro* cell-free system that there may be a considerable "species barrier" reducing the probability that CWD will affect humans. It was shown that PrPres associated with chronic wasting disease (PrP^{CWD}) from elk, mule deer or white-tailed deer was able to readily induce substantial conversion of recombinant cervid PrPsen molecules from any of these three species to the protease-resistant state. In the same system, CWD-associated PrPres was shown to convert human PrPsen but at a much lower efficiency: more than 14-fold lower efficiency than inter-cervid conversion reactions and more than five-fold lower than conversion of human PrPsen by PrPres from the brains of humans with CJD (Raymond *et al.*, 2000). While encouraging, interpretation of this study is complicated by the fact that conversion of human PrP^C by PrP^{BSE} and PrP^{Sc} from sheep were of similar efficacy, both being more than 10-fold less efficient compared with corresponding homologous conversions) and one of these appears to be orally transmissible to humans (BSE) while the other (scrapie) appears not to be (Raymond *et al.*, 2000). In previous experiments PrP^{BSE} had showed 10-fold greater conversion efficacy for bovine PrPsen than for human codon 129-M (methionine) PrPsen and 30-fold greater conversion efficacy than for human codon 129-V (valine) PrPsen, while ovine PrP^{Sc} showed five-fold greater conversion efficacy for ovine PrPsen than for human 129-M PrPsen and eight-fold greater conversion than for human 129-V PrPsen (Raymond *et al.*, 1997).

227. Results of recent work in transgenic mice expressing human PrP (see paragraph 71), in which transmission of CWD from elk by intracerebral inoculation failed, was considered to "strongly suggest" a species barrier to transmission of elk CWD to humans (Kong *et al.*, 2004).

Potential risk from consuming cervid products

Velvet antler

228. Limited studies to date indicate risk from this product may be very low. No CWD-specific PrP accumulation was detected in a sample of velvet from an elk stag which developed clinical CWD about three months later; there were severe brain lesions and extensive CWD-specific PrP staining in both the brain and peripheral lymphoid tissue of the stag (Kahn *et al.*, 2004).

Consumption of venison and other parts of the animal

229. PrP^{CWD} has not been detected in muscle tissue from infected cervids (Spraker *et al.*, 2002c). However, it has been recommended by the World Health Organisation that no parts or products of any animal known to be CWD-positive should be consumed (WHO, 2000). Public health authorities in the USA and Canada have indicated agreement with this (Canadian Food Inspection Agency, 2003; Chronic Wasting Disease Alliance, 2004). It has been suggested that if a harvested cervid is being tested for CWD, the test results should be awaited before the meat is eaten (Wisconsin Department of Agriculture, Trade and Consumer Protection, 2002). Authorities in North America have widely advised that (a) tissues likely to contain the greatest amount of CWD agent in infected cervids, including the brain, spinal cord, lymph nodes, spleen, tonsils and eyes, should not be consumed from any harvested deer; (b) meat should be boned out and fat and connective tissue removed (which would also remove lymph nodes); and (c) hunters should avoid eating meat from deer or elk which look sick or which test positive for CWD (Buege, 2002; Chronic Wasting Disease Alliance, 2004; Williams *et al.*, 2002; Wisconsin Department of Agriculture, Trade and Consumer Protection, 2002; Belay *et al.*, 2004).

Potential risk from handling and processing cervids

230. In order to minimise any potential risk from exposure to the agent of CWD, hunters, meat processors and taxidermists handling cervid carcasses are advised to wear latex or rubber gloves when handling or dressing cervids from CWD-endemic areas, to minimise handling of brain and spinal cord, and to thoroughly wash knives and other implements after use on deer or elk carcasses (Belay, 2004; Williams *et al.*, 2002). It has been suggested that the risk of “build-up” of infectious CWD agent in a venison processing plant would be unlikely (Buege, 2002).

Potential risk from disposal of carcasses and subsequent contamination of ground/water/air

231. In 2002, a risk analysis was produced on disposal of deer from Wisconsin in municipal solid landfills. It was noted that it is not known how much infected material a human (or animal) must consume or be exposed to in order to be infected with CWD. The report took into account the probable species barrier for transmission to humans (Raymond *et al.*, 2000). It was noted that the CWD agent is hydrophobic and likely to adhere to organic materials within a landfill, taking several months to move through the landfill, and that any infectivity exiting the landfill would be captured in the landfill effluent. If effluent was transferred to a wastewater plant (rather than recirculated in the landfill) the agent would be expected to partition with the sludge fraction, which would be diluted greatly with other solids and mixed with nine inches (22.5 cm) of topsoil, providing “an extremely large dilution factor.” It was concluded that there was no significant risk to human health from disposing of deer infected with CWD in properly constructed landfill sites (Olander, 2002).

TABLES

Table 1: Summary of results of CWD transmission experiments

DONOR SPECIES	RECIPIENT SPECIES	SUCCESS / FAILURE	INCUBATION PERIOD	CONFIRMED BY?	NOTES	REFERENCES
Intracerebral Inoculation						
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	17 months and 21.5 months (two individuals)	Histopathological lesions of spongiform encephalopathy	--	Williams, Young & Marsh, 1982; Williams & Young, 1992
Mule deer <i>Odocoileus hemionus</i>	White-tailed deer <i>Odocoileus hemionus</i>	Successful	Not available	Study is ongoing	Full details not available at this time	Hamir, 2004; A.N. Hamir, pers. comm. 2004
Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	White-tailed deer <i>Odocoileus hemionus</i>	Successful	Not available	Study is ongoing	Full details not available at this time	Hamir, 2004; A.N. Hamir, pers. comm. 2004
White-tailed deer <i>Odocoileus hemionus</i>	White-tailed deer <i>Odocoileus hemionus</i>	Successful	Not available	Study is ongoing	Full details not available at this time	Hamir, 2004; A.N. Hamir, pers. comm. 2004
Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	Fallow deer <i>Dama dama</i>	No transmission to date (after two years)	Not applicable	Study is ongoing	Full details not available at this time	Hamir, 2004
White-tailed deer <i>Odocoileus hemionus</i>	Fallow deer <i>Dama dama</i>	No transmission to date (after two years)	Not applicable	Study is ongoing	Full details not available at this time	Hamir, 2004
Mule deer <i>Odocoileus hemionus</i>	Domestic goat	Successful; clinical signs developed	Six years	Histopathology, Western blot	Details not published	Williams & Young, 1992; E.S. Williams, pers. comm., 2004
Mule deer <i>Odocoileus hemionus</i>	Domestic cattle	Successful to five of 13 individuals	22 months to five years	PrPres deposition in CNS detected by IHC and Western blot	--	Hamir <i>et al.</i> , 2001; Hamir <i>et al.</i> , 2004a
Mule deer <i>Odocoileus hemionus</i>	Domestic sheep	Successful to one individual	35 months to euthanasia (Hamir, pers. comm.)	Histopathological lesions, PrPres detected	Full details not available at this time	Hamir <i>et al.</i> , 2003a, Hamir, 2004
Mule deer <i>Odocoileus hemionus</i>	Squirrel monkey <i>Saimiri sciureus</i>	Successful	--	Histopathology (E.S. Williams, pers. comm., 2004)	Details not published	Williams & Young, 1992 (Marsh, R.F., unpublished data)
Mule deer <i>Odocoileus hemionus</i>	Ferret <i>Mustela putorius fero</i>	Successful	17-21 months	--	Complete details not published	Williams, Young & Marsh, 1982
Mule deer <i>Odocoileus hemionus</i>	Ferret <i>Mustela putorius fero</i>	Successful	14 to 19 months	Histological lesions; presence of PrP by IHC, ELISA and Western blot	Developed clinical disease	Sigurdson <i>et al.</i> , 2003
Mule deer <i>Odocoileus hemionus</i>	Ferret <i>Mustela putorius fero</i>	Successful	17 to 21 months	Histological lesions.	--	Bartz <i>et al.</i> , 1998

Ferret <i>Mustela putorius fero</i>	Ferret <i>Mustela putorius fero</i>	Successful	8-9 months (first passage); five months (second and subsequent passage)	Histological lesions.	--	Bartz <i>et al.</i> , 1998
Mule deer <i>Odocoileus hemionus</i>	Common raccoon (<i>Procyon lotor</i>)	FAILED to date (to five years)	Not applicable	Not applicable	Study is ongoing	Hamir <i>et al.</i> , 2003c, Hamir, 2004
Mule deer <i>Odocoileus hemionus</i>	Laboratory mouse	FAILED, several experiments	Details not published	Details not published	Details not published	Williams & Young, 1992
Mule deer <i>Odocoileus hemionus</i>	Laboratory mouse	Successful	More than 500 days	Neuropathology, including perivascular PrP accumulation	Disease developed in "a very few mice"	Bruce <i>et al.</i> , 2000
Laboratory mouse	Laboratory mouse	Successful	Variable depending on the PrP genotype of the mice	Predominantly vascular PrP pathology.	From mice which developed clinical signs. Successful into all strains of mice	Bruce <i>et al.</i> , 2000
Mule deer <i>Odocoileus hemionus</i>	Syrian (golden) hamster <i>Mesocricetus auratus</i>	FAILED, several experiments	Not applicable	No further details available	No further details available	Williams & Young, 1992; Williams <i>et al.</i> , 1992
Mule deer <i>Odocoileus hemionus</i>	Syrian (golden) hamster <i>Mesocricetus auratus</i>	FAILED (experiment terminated after one year)	Not applicable	- No further details available	No further details available	Bartz <i>et al.</i> , 1998
Ferret <i>Mustela putorius fero</i> (second passage)	Syrian (golden) hamster <i>Mesocricetus auratus</i>	Successful	132 to 187 days	Western blot on whole brain homogenate	Transmission into three of 24 individuals	Bartz <i>et al.</i> , 1998
Ferret <i>Mustela putorius fero</i> (fourth passage)	Syrian (golden) hamster <i>Mesocricetus auratus</i>	Successful	152 +/- 44 days (range 100-239 days)	Western blot on whole brain homogenate	Transmission into 16 of 20 individuals	Bartz <i>et al.</i> , 1998
Syrian (golden) hamster <i>Mesocricetus auratus</i>	Syrian (golden) hamster <i>Mesocricetus auratus</i>	Successful	Approximately 50-60 days	Western blot on whole brain homogenate	100% transmission from hamsters showing clinical signs	Bartz <i>et al.</i> , 1998
Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	Transgenic mice expressing human PrP	FAILED (no transmission by 386 days post inoculation)	Not applicable	Full details not available at this time	Full details not available at this time	Kong <i>et al.</i> , 2004
Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	Transgenic mice expressing cervid PrP	Successful	Average 118 days	--	Full details not available at this time	Kong <i>et al.</i> , 2004

Oral inoculation						
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	Detectable PrP ^{res} staining in lymphoid tissues by 42 days.	Confirmed by IHC	--	Sigurdson <i>et al.</i> , 1999
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	PrP ^{res} in cervical lymph nodes by three months, in parasympathetic vagal nucleus by six months	Confirmed by IHC. Histopathological lesions in the parasympathetic vagal nucleus by 16 months	Minimum about 16 months to clinical signs	Williams & Miller, 2000
Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	Rocky Mountain elk <i>Cervus elaphus nelsoni</i>	Successful	Approximately 12 to 34 months to onset of clinical disease	Histology, IHC, ELISA	Details not published	Williams & Miller, 2002; E.S. Williams, pers. comm., 2004
White-tailed deer <i>Odocoileus hemionus</i>	White-tailed deer <i>Odocoileus hemionus</i>	Successful	Details not published	Histology, IHC, ELISA	Details not published	Williams, 2001; E.S. Williams, pers. comm., 2004
Mule deer <i>Odocoileus hemionus</i> (E.S. Williams, pers. comm., 2004)	Moose (<i>Alces alces</i>)	Transmission detected in one animal	Less than 465 days	PrP detected by IHC of the obex	Infected animal died from unrelated causes. No further details available at this time.	T. Kreeger, pers. comm., 2004
Mule deer <i>Odocoileus hemionus</i>	Domestic cattle	No transmission detected by six years	Not applicable	Study is ongoing	Study is ongoing	Williams, 2001; Belay <i>et al.</i> , 2004
Deer (species not stated)	Ferret <i>Mustela putorius fero</i>	No transmission	Not applicable	--	Full details not available at this time	Perrott <i>et al.</i> , 2004
Ferret <i>Mustela putorius fero</i>	Ferret <i>Mustela putorius fero</i>	Successful	Transmission	PrP ^{CWD} was detected	Full details not available at this time	Perrott <i>et al.</i> , 2004
Intraperitoneal inoculation						
Ferret <i>Mustela putorius fero</i>	Ferret <i>Mustela putorius fero</i>	Successful	Transmission	--	Full details not available at this time	Perrott <i>et al.</i> , 2004
Contact						
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	Less than one year to detection by tonsillar biopsy	Confirmed <i>post-mortem</i>	No clinical signs. Infection confirmed in two of 10 deer within one year.	Miller <i>et al.</i> , 2004
Paddock contaminated with excreta						
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	Less than one year to detection by tonsillar biopsy	Confirmed <i>post-mortem</i>	No clinical signs. Infection confirmed in one of nine deer within one year	Miller <i>et al.</i> , 2004

Paddock containing decomposed (skeletonised) carcass						
Mule deer <i>Odocoileus hemionus</i>	Mule deer <i>Odocoileus hemionus</i>	Successful	Less than one year to detection by tonsillar biopsy	Confirmed <i>post-mortem</i>	No clinical signs. Infection confirmed in three of 12 deer within one year.	Miller <i>et al.</i> , 2004

Table 2: Summary of PRNP gene differences between species and polymorphisms within species for the known natural hosts of CWD

SPECIES	Codon 95	Codon 96	Codon 116	Codon 132	Codon 138	Codon 226
Elk <i>Cervus elaphus nelsoni</i>	Q	G	A	L/M	S	E
Mule deer <i>Odocoileus hemionus</i>	Q	G	A	M	S (functional)/ N (pseudogene)	Q
White-tailed deer <i>Odocoileus virginianus</i>	Q/H	G/S	A/G	M	S (functional)/ N (pseudogene)	Q

Based on data from Cervenáková *et al.*, 1997, O'Rourke *et al.*, 1998a, O'Rourke *et al.*, 1999, Raymond *et al.* 2000, Johnson *et al.* 2003, Brayton *et al.*, 2004, O'Rourke *et al.*, 2004.

Table 3: Comparison of PRNP sequence variations from some North American and European cervid species

SPECIES	Codon 59	Codon 95	Codon 96	Codon 98	Codon 116	Codon 132	Codon 138	Codon 226	GenBank Accession No.
Elk <i>Cervus elaphus nelsoni</i>		Q	G	T ⁻	A	L/M	S	E	AY237542, AF156182
Mule deer <i>Odocoileus hemionus</i>		Q	G	T ⁻	A	M	S (functional)/ N (pseudogene)*	Q	AF009181
White-tailed deer <i>Odocoileus virginianus</i>		Q/H	G/S	T ⁻	A/G	M	S (functional)/ N (pseudogene)*	Q	AY275711, AY275712 AY286008, AF091560
European moose <i>Alces alces alces</i>		Q	G		A	M	S	Q	AY639095
Shiras moose <i>Alces alces shirasi</i>		Q	G		A	M	S	Q	AY225484
Fallow deer <i>Dama dama</i>		Q	G		A	M	N [^]	E	AY639094, AY286007
Roe deer <i>Capreolus capreolus</i> (Sweden)		Q	G		A	M	S	Q	AY639096
Roe deer <i>Capreolus capreolus</i> (Italy) [§]		Q	G		A	M	S	Q	
Reindeer <i>Rangifer tarandus tarandus</i>		Q	G		A	M	S	Q	AY639093
Reindeer <i>Rangifer tarandus</i>							N [^]		
Red deer <i>Cervus elaphus elaphus</i> (Italy) [§]	G/S			T/A				Q/E	
Indian Muntjac <i>Muntiacus muntjak</i>				S [~]					
Wapiti <i>Cervus elaphus canadensis</i>				T [~]					
Dybowski deer <i>Cervus Nippon dybowsii</i>				T [~]					

Based (including GenBank accession numbers) on Simonsson, Klingeborn & Linne, 2004, with additional data [§] from Peletto *et al.*, 2004.

Peletto *et al.* (2004) also found in roe deer and red deer at codon 136 a polymorphism gct/gcc and in red deer at codon 79 a polymorphism ccc/cct

* Information regarding functional and pseudogenes in *Odocoileus* spp. from Brayton *et al.*, 2004 and O'Rourke *et al.*, 2004

[^] Unpublished data referred to in O'Rourke *et al.*, 2004 and confirmed by K. O'Rourke, pers. comm., 2004.

⁻ K. O'Rourke, pers. comm., 2004

[~] Wopfner *et al.* (1999) [At human codon 95]; this is equivalent to cervid codon 98 (K. O'Rourke, pers. comm., 2004)

Note: A polymorphism in *Odocoileus* spp. deer at codon 151 (C/R) is reported by Heaton *et al.* (2003) and in white-tailed deer *Odocoileus virginianus* by Johnson *et al.*, (2003). In studies in which the functional gene and pseudogene have been separated, this polymorphism has been found only in the pseudogene (Brayton *et al.*, 2004; K. O'Rourke, pers. comm., 2004)

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