

CHRONIC WASTING DISEASE
Review of research published since November 2004

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1) INTRODUCTION

1. At SEAC 85 (November 2004), SEAC considered the possible animal and human health implications of chronic wasting disease (CWD) in UK deer. The committee was provided with a comprehensive review of published as well as some unpublished research on CWD compiled by the Wildlife Information Network (WIN) (*WIN 2004*). This paper summarises experimental and epidemiological research published subsequently and provides an update to the WIN review (*Annex 1 of SEAC paper 85/2*). This paper follows a similar format to the original review for ease of comparison. The methods used to identify new published research were consistent with the methods used in the WIN review. Literature was identified by the following methods:

- Searches on **PubMed** (<http://www.pubmed.gov>) were performed using the following keywords; “CWD”, “Chronic Wasting Disease”. “transmissible spongiform encephalopathy”, “deer” and “elk”. Key authors, were also used.
- Many of the search engines used by the WIN were not available during the review, however a number of suitable alternative sites were identified:
 - **ingentaconnect** (<http://www.ingentaconnect.com/>),
 - **scirus** (<http://www.scirus.com>),
 - **sciencedirect** (<http://www.sciencedirect.com/>),
 - **Agricola** (<http://agricola.nal.usda.gov/>),
 - **ISI web of knowledge** (<http://go.isiprducts.com>).

Web searches with Google were also used. Keywords and methods were the same as those employed with PubMed.

2) AETIOLOGY AND POSSIBLE ORIGINS

Aetiology

2. The literature reviewed by WIN suggested that the TSE agent associated with CWD is different from the agents associated with bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy (TME), Creutzfeldt-Jakob disease (CJD) and known scrapie strains (**WIN 2004**). No new data has been published which contradicts this conclusion.

Strains of CWD

3. A study by Browning *et al.* examined the possibility that more than one strain of CWD agent may exist (**Browning et al, 2004**). Transgenic mice expressing the cervid prion protein (PrP) gene were intracerebrally (ic) inoculated with brain homogenate from captive and free-ranging elk or mule deer with clinical CWD as well as pooled samples from diseased animals. The disease incubation times and neuropathological features in infected mice and the biochemical properties of PrP^{CWD} derived from these mice were similar for each inoculum suggesting the same strain of TSE agent was responsible for CWD. However, the PrP^{CWD} distribution in the brain of mice inoculated with one mule deer brain homogenate was different compared with the mice given other inocula. The authors suggest this may be evidence of a different CWD strain. Further work is needed to characterise and confirm this finding.

Possible Origins of CWD

Scrapie

4. The WIN review discussed the possible origins of CWD and suggested that it was possible that a scrapie agent may have adapted to a form that was able to infect cervids as ic inoculation of elk with scrapie resulted in central nervous system (CNS) lesions indistinguishable from CWD. In a recent review Williams noted there were features common to both CWD and scrapie including the early widespread distribution of disease-associated PrP^{Sc} in lymphoid tissues with the later involvement of the CNS (**Williams 2005**). However, there is no evidence of natural transmission of scrapie from sheep to cervids.

3) KNOWN GEOGRAPHICAL DISTRIBUTION AND TIMELINES OF SPREAD

In farmed cervids

USA

5. The WIN review noted that CWD was present in farmed cervids in Colorado, Kansas, Minnesota, Montana, Nebraska, Oklahoma, South Dakota and Wisconsin. However, in March 2005 CWD was detected for the first time in farmed cervids in the eastern United States. CWD was confirmed in five white tailed deer from two herds in Oneida County, New York State (**O'Rourke 2005**). The source of infection has not been determined but infection from wild deer had not been ruled out. Although not a new discovery, it should also be noted that CWD in farmed herds was also found in Wyoming in 1978, this information was omitted from the WIN review (**Williams et al, 2002**).

Outside North America

6. CWD had previously been reported in farmed elk imported into Korea from Canada in 1994 and 1997. As a result of infection the elk were slaughtered in 2001 along with Korean co-habiting elk, however, 43 of the potentially infected elk could not be traced. In November 2004 a further elk was found to have CWD. It is not known whether this was one of the 43 elk not traced from the infected stock (**Kim et al, 2005**). Tests are being performed to determine whether this new case of CWD may have been transmitted vertically or horizontally.

In free-ranging cervids

USA

7. In April 2005, a month after the first confirmation of CWD in the New York state farmed cervid population, a free ranging white tailed deer in the state was identified with the disease (**NYS 2005a**) with a further case detected in May 2005 (**NYS 2005b**). Peterson suggests these results may provide evidence for a link between the cases of CWD in farmed deer in the same state (**Peterson 2005**). New York State does not share a border with any other affected state.
8. In September 2005, the West Virginia Division of Natural Resources announced this state's first case of CWD, a 'road kill' white tailed deer (**WVDNR 2005**). A suggestion had been made that 'road kill' deer are used as a way of increasing the efficiency of surveillance programmes as CWD infected deer have a relatively high vulnerability to vehicle collisions (Krumm et al, 2005). Since the first case, seven further white tailed deer have been determined to have CWD with the latest diagnosed in April 2006 (**WVDNR 2006**). As with New York State, West Virginia does not border any state identified as having CWD in its cervid population.
9. In January 2006 the first free ranging case of CWD was identified in a white tailed deer in Kansas (**KDWP 2006**).

Canada

10. The first case of CWD infectivity in the wild mule deer in Alberta was reported in September 2005 (**GOA 2005**). Alberta was already identified as having CWD in its farmed deer population.

Outside North America

11. Surveillance programmes for 'free-ranging' cervids have been reported in Germany (**Schettler et al, 2006**), south-eastern Belgium (**Roels et al, 2005**), Denmark, Hungary, Slovenia, Finland (**EC 2004, EC 2005**), Hokkaido Island Japan (**Kataoka et al, 2005**) and the UK (**PJ Burke, per. Comm., 2006**). There is no evidence of TSEs in the cervids tested from any of these countries (see section 9).

4) HOST RANGE

Known natural hosts

12. The known natural hosts of CWD were previously reported by WIN (2004) as mule deer (*Odocoileus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*) and the Rocky Mountain elk (*Cervus elaphus nelsoni*). The disease has also occurred naturally in hybrids of these breeds. In September 2005 the Colorado Division of Wildlife confirmed that a free ranging bull moose (*Alces alces*) killed for game had tested positive for CWD (**CDW 2005; Peterson 2005**). Although this was the first time that CWD had been identified in moose it was not unexpected as experimental work had shown that this species can develop CWD after oral inoculation of infectious material (**Williams 2005**).

Experimental transmission by intracerebral inoculation

13. In addition to those species previously stated as being susceptible to ic inoculation of CWD-affected brain material (mule deer, white tailed deer, ferrets, American mink, goat, cattle, sheep), transmissions to transgenic mice expressing cervid PrP, but not wild type mice, (**Browning et al 2004**) and two squirrel monkeys (**Marsh et al, 2005**) have now been reported.

14. Preliminary findings from a study of transgenic mice expressing human PrP suggested such mice were resistant to CWD after ic inoculation of CWD on the basis of a lack of clinical signs of infection after 386 days (**Kong et al, 2004**). This study has now been extended with the result that no clinical or pathological signs of transmission were observed after more than 756 days (**Kong et al, 2005**). This is discussed further in paragraph 45.

15. Thirteen cattle calves were ic inoculated with a suspension of brain material from CWD-infected mule deer with three calves kept as controls (**Hamir et al, 2005**). After six years, two of the inoculated animals showed clinical signs of infection. PrP^{res} was found in the CNS of five inoculated animals however, the microscopic lesions expected in spongiform encephalopathy (SE) were subtle in three cases and absent in two cases. The eight remaining inoculated animals and three controls all tested negative for signs of infection. The study suggests that CWD may be weakly transmissible to cattle after ic inoculation.

16. In a follow-up study, six calves were inoculated ic with brain tissue derived from the PrP^{CWD} positive cattle described in paragraph 15 (**Hamir et al, 2006a**). At 16.5 months, all six inoculated cattle showed acute clinical signs of disease resulting in the termination of the experiment. Although microscopical SE-type lesions were not observed in any of the cattle, PrP^{res} was detected in CNS tissues. The authors noted that these findings were similar to those in a study of cattle inoculated with the scrapie agent where, in the absence of SE lesions, cattle showed clinical signs of infection and PrP^{res} was found in CNS tissues. The authors suggest that distinct differences exist between CWD and BSE in cattle enabling clear identification of both diseases if natural transmission of CWD to cattle were to occur.

Experimental transmission by oral inoculation

17. Oral transmission of CWD has been observed in mule deer, white-tailed deer and elk. New experiments examining the genetic susceptibility in elk through oral transmission have been performed and are described in section 8. Preliminary results suggest that elk of the 132MM genotype are highly susceptible to the disease whilst, by comparison, elk of the 132LL genotype appear to be relatively resistant to the disease (***Hamir et al, 2006b***).
18. Two studies of oral inoculation of cattle with brain material from CWD-infected cervids reported in the WIN review continue. In these studies, animals were inoculated orally with a single dose of CWD that would be sufficient to infect a cervid. After more than 7 years, cattle remain healthy displaying no clinical signs of the disease (***Hamir et al, 2005***). The authors suggest that if inoculated cattle have been shown to develop CWD it may be assumed that cattle would require a large dose of inoculum and a long incubation time to develop infection.

Natural transmission by contact/environmental contamination

19. The WIN review reported that moose which had been exposed naturally to CWD agent did not develop CWD, however caution was advised as the number of observations was limited. Since then the Colorado Division of Wildlife identified CWD in a wild moose (***CDW 2005***). It was suggested that the occurrence of CWD in moose may remain low due to the solitary nature of these animals which generally only roam in cow-calf pairs, inhibiting widespread transmission of the disease.

5) CWD IN THE KNOWN NATURAL HOSTS

Incubation period in cervids

20. Experimental studies suggest that variations in the PrP gene may account for differences in CWD incubation periods in elk (***Hamir et al 2006b***). Elk of the 132MM genotype (n= 2) developed clinical signs of CWD with a mean incubation period of 23 months following oral inoculation, similar to that previously reported in the WIN review in free-ranging and farmed elk. However, elk of the 132LM genotype (n= 2) have a longer mean incubation period of 40 months. Elk of the 132LL genotype did not develop clinical signs of CWD four years after oral inoculation of CWD infected brain material. The authors suggest that the 132LL genotype confers either a reduced susceptibility to the disease or an increased incubation time.

Pathological findings

PrP deposition

21. The WIN review reported variations in the deposition of PrP^{CWD} between cervid species. Amyloid plaques are observed in brain tissue of most clinically-affected white-tailed deer and in some mule deer, however these plaques are not always obvious in brain sections from elk.

22. In a study of PrP^{CWD} distribution in the obex and cranial lymphoid tissues of the elk, variable patterns of PrP^{CWD} deposition were observed between animals in contrast to the relatively consistent patterns seen in mule deer (**Spraker et al, 2004**). In elk, four distinct distributions of PrP^{CWD} were observed within the parasympathetic region of the dorsal motor nucleus of the vagus nerve and adjacent nuclei. After immunohistochemical assessment of the medulla oblongata in 226 CWD affected elk, 155 were found to have PrP^{CWD} deposits in both brainstem and lymphoid tissues. Twenty eight had PrP^{CWD} only in the brainstem while 43 had only the lymphoid tissue affected. Genotyping of the elk did not reveal any correlation between genotype and the pattern of distribution of PrP^{CWD}.
23. Research reported in the WIN review suggested that PrP^{CWD} was not detectable in the skeletal muscle of CWD positive mule deer by immunohistochemical methods (**Spraker et al, 2002c**). However, recent research using transgenic mice expressing the cervid PrP as bioassays showed that CWD infectivity was detectable in the skeletal muscles of CWD infected deer (**Angers et al, 2006; Quirk 2006**).

Inhibitors of PrP^{CWD} formation

24. Using a transformed cervid cell line that produces PrP^{CWD}, two inhibitors of PrP^{CWD} accumulation were identified (**Raymond et al, 2006**). Both pentosan polysulfate and indium (III) meso-tetra(4-sulfonatophenyl)porphine chloride blocked PrP^{CWD} accumulation in the cells. The authors suggest that these findings highlight the effectiveness of transformed cervid cells in a screening assay for inhibitors to PrP^{CWD} accumulation.

6) DIAGNOSIS

Immunohistochemistry

25. A comparison of two automated immunohistochemistry platforms has been made to determine their suitability for diagnosing CWD (**Braszler, 2006**). Both Ventana NexES and DakoCytomation Autostainer Universal Staining System use the same antiprion protein monoclonal primary antibody. Ensuring identical tissue brainstem and lymphoid tissues were used, there was 100% agreement between the two systems when diagnosing CWD in white tailed deer. An identical result was observed for scrapie infected sheep. For mule deer an agreement of 98.6% using lymphoid tissue and 99.9% using brainstem was seen between the systems.

Immunoblotting

Western blot

26. The western blot (WB) assays have been used to detect PrP^{CWD} in the brain of asymptomatic and symptomatic cervids infected with CWD. Huang compared analysis with or without PrP^{Sc} concentration using sodium phosphotungstic acid (PTA) precipitation. Two sample preparation methods were used to enhance the sensitivity of CWD detection in WB assays (**Huang 2005**). All six PrP^{CWD} positive samples were determined as such using PTA

precipitation whilst only five out of six samples were classified as positive without PTA precipitation. There were no false positives using either procedure (n= 35). Serial dilution of PTA precipitated samples showed that the use of PTA increases the detection limit of the WB procedure by approximately 100 times.

Choice of tissues for testing

27. It has been suggested that tonsillar follicles collected using the dorso-lateral method of sampling may provide an effective pre-necropsy method of identifying cervids at the pre-clinical stage of CWD infection for prevalence studies (**Schuler et al, 2005**).

7) EPIDEMIOLOGY AND TRANSMISSION

From enclosures previously used by infected animals

28. Research reviewed in the WIN report showed that healthy cervids could be infected with CWD by grazing on pastures contaminated with excreta from infected cervids or with the carcasses of diseased cervids. This provided evidence for environmental transmission of CWD.

29. A more recent experimental study examined the potential for soil to serve as a reservoir for TSEs by examining the interaction of PrP^{Sc} (from an adapted transmissible mink encephalopathy agent) with common soil minerals (**Johnson et al, 2006**). It was demonstrated that substantial PrP^{Sc} could be adsorbed by the clay minerals, kaolinite and montmorillonite as well as quartz. In addition, significant adsorption to whole soil samples was found. Furthermore, there was a strong interaction between PrP^{Sc} and montmorillonite. To determine whether prions remained infectious in soil, clay samples experimentally contaminated with an inoculum were inoculated into hamsters. Symptoms developed in these animals at approximately the same time as those injected with directly with the inoculum. The study suggests that TSE infectivity released into soil may be preserved in a bioavailable form which may contribute to disease transmission. The authors suggest this study adds to the evidence for environmental transmission of CWD.

Epidemiology and spread in free-ranging cervids

30. In a study of free ranging mule deer in north central Colorado, temporal, spatial, and demographic factors all appear to contribute to the marked heterogeneity in disease prevalence in the cervid population (**Miller et al, 2005**). Prevalence of CWD was higher on winter ranges, the author suggesting that the static nature of the animals on these ranges would increase transmission. In areas where disease was endemic there were trends of increasing prevalence seen over a seven year period.

31. In a study to evaluate the potential influence of the movement patterns of free ranging mule deer on the spatial distribution of CWD in north central Colorado, spatial differences CWD prevalence were partly related to deer movements (**Conner et al, 2004**). Migration movements of animals had the

most influence on transmission of the disease with dispersal movements showing little influence.

32. Correlations between the prevalence of CWD in cervids in north central Colorado and the geographical location, gender and land use were examined to assess the potential influence of human alteration of landscapes on the prevalence of CWD in mule deer (**Farnsworth et al, 2005**). Although a higher prevalence of CWD in male deer was found in developed landscapes, the three study sites used showed a high degree of variation suggesting that there was no clear correlation between alternations in CWD prevalence and human activity on landscapes.

8) SUSCEPTIBILITY WITHIN THE KNOWN NATURAL HOSTS

Genetics in Rocky Mountain elk (*Cervus elaphus nelsoni*)

33. Preliminary research has showed that when scrapie was inoculated into elk, those of the 132MM genotype developed the disease first with genotype 132LM following at a later time (**Hamir et al, 2004**). In an extension to this study, elk of the 132MM (n= 2), 132LM (n= 2), 132LL genotype (n= 4) were orally challenged with brain material from CWD infected elk (**Hamir et al, 2006b**). Mean incubation period was 23 months post-inoculation for elk of the 132MM genotype and 40 months for elk of the 132LM genotype. The remaining elk of the 132LL genotype displayed no clinical signs at 4 years post-inoculation suggesting that LL at codon 132 causes either a marked reduction in susceptibility to infection by the oral route and / or have a prolonged incubation time.

Genetics in mule deer (*Odocoileus hemionus*)

34. DNA sequencing of the PrP gene in over 1400 free-ranging mule deer suggested that polymorphisms at codon 225 may influence susceptibility to CWD (**Jewell et al, 2005**). In this study, the probability of a mule deer with the 225SS genotype being infected with CWD was 30 times higher than that for a deer of genotype 225SF (animals of the 225FF genotype comprised < 0.2% of the study population).

Effect of sex and age specific susceptibility?

35. Gender and age related differences in CWD prevalence have been reported in free ranging mule deer (**Miller et al 2005**). The prevalence of CWD amongst male deer was found greatest between 5 and 6 years of age (n= 1772, 290 positive). No such age related pattern was observed among female mule deer. The prevalence of CWD in male deer was higher than that of female for all age ranges apart from 1-2 year olds. The author suggests that mating and social habits of the male deer effect prevalence of CWD. In another study, the prevalence of CWD was again found to be higher in the male compared with the female mule deer population (sample numbers not reported) (**Farnsworth et al, 2005**).

9) ANIMAL HEALTH CONCERNS

Potential risk to other Cervidae

36. Since CWD is known to occur in a wide range of North American cervid species, it is possible that other species of cervids elsewhere in the world may also be susceptible to CWD. For this reason, surveys of TSEs in non-North American cervids have been conducted.

Surveillance studies

Sika deer (*cervus nippon*)

37. In the Tokachi district of Hokkaido Japan, surveillance for CWD was performed on 136 free-ranging sika deer. Western blot analysis was used on tissue samples to detect the presence of PrP^{CWD}. No cases of CWD infection were detected in this population (**Kim et al, 2005**).

Common European deer species – red deer (*Cervus elaphus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), moose (*Alces alces*) and reindeer (*Rangifer tarandus*).

38. Free-ranging red and roe deer in south-eastern Belgium were tested for CWD (**Roels et al, 2005**). Spleen samples taken from 866 roe or red deer were analysed for the presence of PrP^{CWD} using an enzyme-linked immunosorbent assay of Bio-Rad and verified by immunohistochemistry. All the animals tested were negative for CWD infection.

39. Free ranging roe, red and fallow deer from Germany were examined between 2002 and 2005 for the presence of CWD (**Schettler et al, 2006**). In total 7304 deer were tested (4255 roe, 1445 red, and 1604 fallow deer). All the animals tested were negative for TSE infection.

40. Surveillance in 2004 showed no evidence of TSE infection in cervid from Denmark (9 roe, 1 fallow deer). Similarly in 2004 and 2005 monitoring in Slovenia (28 roe, 42 red, 50 fallow deer and 1 elk), Hungary (69 roe, 2 red and 4 fallow deer) and Finland (6 roe, 24 white tailed deer, 385 farmed reindeer, 16 wild reindeer and 43 moose) found no evidence of TSE in the surveyed animals (**EC 2004, EC 2005**).

41. In the UK over 600 roe, fallow and red deer from the royal parks and New Forest were tested by Bio-Rad ELISA and immunohistochemistry for TSE infection in 2004. All the animals tested negative (**Defra 2005**).

Potential risk to domestic cattle & sheep

42. No cases of natural transmission of CWD to livestock or other non-cervid species living have been identified in CWD endemic areas. However, CWD has been transmitted by i.c. inoculation in cattle both on primary and secondary passage of brain homogenate from CWD infected cervid (see paragraph 16).

10) HUMAN HEALTH CONCERNS

43. Epidemiological research reviewed in the WIN report provided no evidence of transmission of CWD to humans from consumption of venison, although studies were very limited. No further epidemiological investigations have been published.

Laboratory studies

44. Laboratory studies have investigated the potential susceptibility of humans to CWD. CWD was transmitted to two squirrel monkeys inoculated with brain tissue from a CWD-infected mule deer with clinical signs of infection observed at 31 and 34 months post inoculation at which point the animals were sacrificed (**Marsh et al, 2005**). Histological examination confirmed neurodegeneration consistent with CWD infection.

45. In a preliminary study, no transmission of CWD was observed 386 days after transgenic mice expressing human PrP were inoculated with brain homogenate from an elk with CWD (**Kong et al, 2004**). In an extension to this study, two lines of humanised transgenic mice inoculated with brain homogenate from an elk with CWD failed to develop clinical signs of TSE 657 and 756 days post inoculation (**Kong et al, 2005**). In contrast, transgenic mice expressing the cervid prion protein gene became infected after 118 days post inoculation.

46. A comparative analysis of the PrP^{Sc} isolated from samples from cervids with CWD, sCJD patients and sCJD patients that had consumed meat potentially from CWD infected cervids was carried out using histopathological, immunochemical and conformation dependent assays (**Xie et al, 2006**). The analysis showed distinct differences between the cervid and human samples.

Potential risk from consuming cervid meat

47. CWD infectivity has been detected using cervid transgenic mice as bioassays and PrP^{CWD} in the skeletal tissues of CWD infected cervids (**Angers et al, 2006**).

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