EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Scientific Steering Committee

Chronic wasting disease

AND TISSUES THAT MIGHT CARRY A RISK

FOR HUMAN FOOD AND ANIMAL FEED CHAINS

REPORT

1 MANDATE

The TSE/BSE ad hoc Group was invited to prepare a scientific report to serve as the basis for addressing the following questions about chronic wasting disease (CWD):

1. Do there exist scientific bases to exclude certain tissues from animals that carry a CWD risk from the human food and animal feed chain? If so, which tissues could pose a risk? Is there a basis for defining SRM?

2. Is there a reason for concern that imports from countries where CWD has been observed pose a risk to animal and/or consumer health in Europe? If yes, what measures are likely to be proposed?

3. Is CWD also occurring elsewhere, e.g. Europe?

2 PREAMBLE

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) or "prion disease" of certain species of native North American deer: mule deer (*Odocoileus hemionus)* and white-tailed deer (*Odocoileus virginianus)* and Rocky Mountain elk (*Cervus elaphus nelsoni*) occurring in captive, farmed and free living populations. This is the only TSE known to affect free-ranging wildlife species. Its reported occurrence other than in North America (NA) is confined to a single case in an elk imported into Korea from NA (Sohn et al, 2002).

The report that follows provides a review of current knowledge and examines scientific data on which judgements can be made to exclude certain tissues of any animal, which might carry a risk for CWD, from human or animal feed chains. If the basis of such judgements can be determined the report will also provide information as to which tissues to identify for exclusion and under what circumstances.

The report necessarily considers current knowledge concerning CWD in NA, the principle geographical region of its occurrence. It addresses any effects of the disease on the uses of the affected Cervidae species and products derived from the species both in NA, Europe and globally, as far as can be determined.

The SSC considers that it would be prudent in the context of this report to examine also what is known about the potential for the occurrence of CWD or other TSE in Cervidae species native or farmed in the continent of Europe. This component of the report will examine evidence relating to the susceptibility of such deer species to develop a TSE and what, if any, surveillance for TSE in deer species has been conducted, or is planned, in Europe.

3 CWD IN NORTH AMERICA

3.1 HISTORICAL PERSPECTIVE OF OCCURRENCE

Chronic wasting disease is a naturally occurring transmissible spongiform encephalopathy (TSE) or prion disease of certain species of native North American deer and elk. Originally diagnosed in captive populations this is the only TSE known to affect free-ranging and captive wildlife species. To date, CWD has been reported a limited number of states in the USA and provinces in Canada and also in Korea (imported animal).

3.1.1 CWD in captive and farmed cervidae

The clinical syndrome of wasting and death in captive deer was first recognised in the late 1960's in the USA in a wildlife facility in northern Colorado (Fort Collins area). Biologists working on natural history and nutritional studies with captive **mule deer** observed the clinical signs and called the syndrome chronic wasting disease (CWD). It was initially thought to be associated with stresses of captivity, nutritional deficiencies, or intoxication. Only after histological studies it was recognised as a spongiform

encephalopathy (Williams and Young, 1980). It was later recognised in a similar wildlife facility in Wyoming (Sybille). The disease was also recognised in Rocky Mountain **elk** from both facilities (Williams and Young, 1982). CWD in **farmed elk** it is thought to have entered **Canada** with elk imported from the United States in the late 1980s. The first case of CWD in farmed elk was diagnosed in 1996. Another infected elk farm was detected in 1998. Alberta (56%) and Saskatchewan (37%) have 71,500 elk ie 93% of the farmed elk population of Canada. The two provinces also account for 77% or 826 elk farms in Canada. (A Dagenais, Pers Com 2003). In 2000-2001, 40 infected elk farms were found in the province of Saskatchewan and 2 infected farms (1 herd of elk and 1 herd of white-tailed deer) in the province of Alberta. There are no obvious linkages with the Saskatchewan focus of CWD and possible sources are still being investigated. A total of 8,300 cervids (99% elk) have been slaughtered for CWD control purposes and 231 CWD infected cervids detected by laboratory testing.

CWD in a mule deer in Canada was first reported at the Toronto Zoo in Ontario in 1976. This animal was probably one of a group transferred from the Colorado Division of Wildlife to the Denver Zoo. CWD was not formally diagnosed at the Denver Zoo though clinical signs shown by some deer there were highly suggestive of CWD

3.1.2 CWD in free-ranging cervidae

The first case of CWD in a free-ranging cervid was found in 1981 in a Rocky Mountain **elk** in Rocky Mountain National Park, Colorado, USA. The first affected **mule deer** diagnosed in a wild population was found in 1984 about 1 mile west of the Colorado Division of Wildlife deer pens where CDW was first observed. (Spraker *et al.,* 1997). In the late 1980s, the disease was diagnosed more frequently (Williams and Young, 1992).

Surveillance of wildlife in Canada has detected 7 cases of CWD in wild deer (6 mule deer and 1 white tailed deer and all in the province of Saskatchewan) in the course of testing 16,495 wild cervids in Saskatchewan, Alberta and Manitoba. These cases may have occurred due to interaction of wild deer with farmed elk. It is also possible that there is a reservoir of infection in free-living deer. However, the prevalence of infection appears to be very low, based on wildlife surveillance to date. Further surveillance is undertaken to accurately estimate the prevalence of CWD in wild deer.

Reviews of CWD should be consulted for additional information on the emergence of the disease and other information on all aspects of the disease (Willams and Young 1992, Williams and Miller 2002, Williams et al. 2002).

3.2 NATURAL HOST RANGE

Only three species of cervidae are known to be naturally susceptible to CWD: mule deer, white–tailed deer and Rocky Mountain elk. One case was originally reported in black tailed deer (*Odocoileus hemionus columbianus*) (Williams and Young 1980), a subspecies of mule deer. Hybrid mule deer X white-tailed deer have also been affected.

Other non domestic ruminants, including moose (*Alces alces),* pronghorn antelope (*Antilocapra americana*), Rocky Mountain bighorn sheep (*Ovis canadensis canadensis),* mouflon (*Ovis musimon),* mountain goats (*Oreamnos americanus*), and a blackbuck (*Antilope cervicapra)* have been in contact with CWD-affected deer and elk or have resided in premises in which CWD had occurred and have not developed the disease (Williams and Young, 1992). Cattle, sheep and goats that have resided in research facilities together with CWD-affected animals for prolonged periods or under field conditions did not develop the disease. These observations of apparent cross-species resistance are supported by molecular studies of Raymond *et al.* (2000)[1]and in vivo studies of Miller *et al*. (2000). However, the in vitro conversion experiments are not necessarily informative about the species barrier *in vivo* and should therefore be interpreted with caution.

Assuming a single (parental) strain of agent, susceptibility of cervids depends on the interaction of this agent with host PrP and other undefined host factors. It may depend also on the ability of the CWD agent to mutate to a version to which the new host is more susceptible (i.e. with a shorter incubation period etc.). The efficiency of interspecies transmission of the TSE's (the species barrier) is considered by some to be dependent on the compatibility of the PrP between donor and recipient. The basis of this 'species barrier' is incompletely understood, but interspecies differences in the amino acid

[1] An important factor controlling interspecies TSE susceptibility is prion protein (PrP) homology between the source and recipient species/genotypes. Raymond *et al* show that the CWD-associated PrP-res (PrP^{CWD}) of cervids readily induces *in vitro* the conversion of recombinant PrP-sen molecules to the protease-resistant state in accordance with the known transmissibility of CWD between cervids. In contrast, PrP^{CWD} –induced conversions of human and bovine PrP-sen were less efficient, and ovine PrP-sen was

sequence of the prion protein and the strain of prions involved play crucial roles (Bosque, 2002). Genetic homology between species confers similarities and divergence in the spatial configuration of the respective protein is an important element of the structural basis of the species barrier (Billeter et al. 1997).

Sequences of the prion protein gene are very similar between certain cervid species (Kaluz et al. 1997, O'Rourke et al. 1998, Cervenakova et al. 1997). In term of prion protein sequences relative to phylogeny, reindeer have convergence with white-tailed and mule deer, whereas in non-cervid ruminants there is divergence from the cervids, particularly so in the case of bovines (Wopfner *et al.,* 1999)[2]. Another phylogenetic analysis suggests that cattle and mule deer have converged with great apes including humans in key areas of their prion protein (Krakauer et al. 1996). It is difficult to draw specific inferences from these data but such studies provide indications as to species in which the PrP gene should be examined in more detail.

Polymorphisms of the PrP gene influence susceptibility to infection and disease phenotype. In Rocky Mountain elk, sequence analysis of the PrP gene showed only a single polymorphism; one amino acid change (*Met* to *Leu*) at codon 132[3]. In over 43 Rocky Mountain elk (genotyped and postive for CWD) it was found that elk, homozygous for PrP codon 132-*Met* (M/M) were over-represented in both free-ranging and farm-raised CWD-affected animals when compared to unaffected control groups. In the same group several heterozygous M/L were positive. Positive elk with the homozygous codon 132 L/L were not found (O'Rourke *et al*, 1999 and Belanchandran personal comm.). Research is continuing into the influence of genetics on susceptibility; there may be an association between PrP genotype and resistance in elk but this has not been recognised (O'Rourke 1999 and unpublished data) .

3.3 EXPERIMENTAL TRANSMISSIBILITY

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intermediate. These results demonstrate a barrier at the molecular level that might limit the susceptibility of the non-cervids species to CWD.

[2] The mammalian prion protein has a high degree of sequence conservation. For comparison, percent divergence of prion proteins is 7,6% between human and domestic cattle; 8,4% between human and sheep; 6,5% between human and dybowski deer; 7,6% between human and wapiti deer (elk); and 2,7% between domestic cattle and sheep (Wopfner et al. 1999).

[3] This position is similar to the one coding for TSE susceptibility in humans.

3.3.1 Transmissibility of CWD

There are certain key questions with respect to transmissibility:

1. Can CWD be transmitted artificially to other species, e.g. sheep, cattle, humans?
2. What is the natural transmissibility to other species ?
3. Can transmissions inform on possible similarities between the agent and BSE, natural scrapie or another know TSE source, or does the evidence suggest a novel source?

Several experimental studies to transmit CWD have been conducted, most by intra-cerebral (IC) inoculation. Whilst such studies provide information on susceptibility to the most efficient means of interspecies transmission, they do not inform on interspecies susceptibility by natural routes of transmission. For the latter oral or other possible natural exposure route studies are considered the most appropriate.

3.3.1.1 Intracerebral inoculation studies:

- Mule deer-CWD has been successfully transmitted to ferrets (*Mustela putorius furo*) by the intracerebral (IC) route (Williams *et al.,* 1982) but failed to transmit to Syrian golden hamsters (*Mesocricetus auratus*) even after multiple attempts (Williams and Young, 1992). Primary transmission into ferrets resulted in an incubation period of 17-21 months. On subsequent passage this shortened with a 5 months incubation period by the third ferret passage. Unlike mule deer-CWD, ferret-passaged CWD was transmissible to Syrian golden hamsters and increasing the number of transmission passes of CWD in ferrets increased the pathogenicity of the agent for hamsters (Bartz *et al*, 1998).

- Transmission of mule deer-CWD to mice is possible, but with a very low efficiency at primary transmission to a panel of mouse strains (RIII, C57BL and VM) and results in incubation periods in excess of 500 days. Serial passage in each mouse strain produced clinical disease in all challenged mouse strain at each passage. The phenotype of disease produced in the mice differed from that of previously strain

typed sources, including scrapie and BSE sources, suggesting that the CWD agent is a unique strain of TSE pathogen (Bruce *et al.,* 2000).

- IC inoculation of mule deer-CWD into squirrel monkeys (*Saimiri sciureus*) was successful in one animal and mink are also susceptible to IC inoculation of mule deer CWD (Williams and Young, 1992).
- IC inoculation into one four months old goat with CWD agent resulted in an incubation time of about 6 years. This is longer than what would be expected with scrapie (Williams and Young, 1992).
- CWD transmission to raccoons (*Procyon lotor)* remains unsuccessful to date at three years after inoculation (Hamir et al., 2003) but a difference in incubation time was observed after IC infection into raccoons when comparing infection with TME (6 months) and scrapie (2 years) in cattle.
- Studies in progress at the National Animal Disease Centre (NADC) have shown preliminary evidence of the transmission of mule deer-CWD to sheep. To date, 1 out of 8 sheep inoculated IC with CWD brain suspension has shown clinical signs at 35 months post inoculation. Histopathological changes indistinguishable from those of scrapie were found and IHC examinations of the brain, tonsil and some lymph nodes proved positive. (Janice Miller, Personal Comm.).
- On-going research on the species barrier is indicating that there is a substantial biological barrier to transmission of CWD from deer to cattle. Preliminary data from experiments in progress in Colorado, Wyoming, and Iowa indicate that only a few calves develop disease after challenge with CWD pathogen from affected mule deer. In 3 calves euthanised between 24 and 27 months post-inoculation (IC), microscopic lesions in the brain were subtle or absent. However, all 3 animals were positive for PrPres by immunohistochemistry (IHC) and Western blot. Three years after challenge, the 10 remaining inoculated cattle were alive and apparently healthy (Hamir *et al*, 2001; Hamir, 2002).

3.3.1.2 Oral and other natural exposure route studies

- Homologous CWD has been transmitted by oral inoculation in mule deer, white-tailed deer, and elk in pathogenesis studies that are nearly complete. In addition,

homologous CWD has been transmitted in elk at a low dose (0.1 g pooled CWD-elk brain) by oral exposure (Williams, pers. Comm.).

- Cattle have been inoculated orally with a brain pool from CWD affected mule deer at the University of Wyoming and have not developed any evidence of transmission more than 5 years following exposure. These studies are scheduled to run for 10 years. In addition, bovine calves have been orally inoculated with CWD brain pools from mule deer and from elk; these calves are being sequentially necropsied and results are not yet available (Williams, pers comm).
- Cattle living in close contact with infected deer and elk have not developed the disease during the first five years of a 10-year study. Twenty-four cattle were housed with resident deer and elk with endemic CWD, in two wildlife research facilities in Wyoming and Colorado. These studies started in 1997 and to date there is no evidence of transmission of CWD to cattle through contact. Control deer have all succumbed to CWD (Williams, 2002).
- Brains from cattle over 5 years of age and from different ranches within an enzootic area of CWD were examined with H&E and IHC stains and all were found negative (Gould *et al*, 2003).

3.3.2 Susceptibility of deer and elk to other TSEs

Scrapie has been successfully transmitted to 3/5 elk after IC inoculation (Hamir et al., 2003). There is apparently no ongoing work at present to attempt transmission of scrapie to mule deer.

3.4 EPIDEMIOLOGY

3.4.1 Descriptive Epidemiological Features

3.4.1.1 Chronology of distribution in the USA

The occurrence of CWD was, for several years after its discovery, noted only in two wildlife research facilities in Colorado and Wyoming. Mule deer supplied to two other research facilities from the Colorado wildlife facility developed CWD. In captive

deer and elk prevalence varies from <1% to nearly 100% depending on the herd size, location, and the origin of animals. It is highest in the captive facilities where CWD has been present for many years. Between June 1986 and May 1997, CWD was the only reported natural cause of adult mortality among Rocky mountain elk held at the wildlife research facility near Fort Collins (Colorado). Of 23 elk that remained in this herd >15 months old, four developed CWD.

In the early 1980's, CWD was recognised in **free-ranging cervids** (Spraker *et al*., 1997). Between March 1981 and June 1995, CWD was diagnosed in 49 free-ranging cervids from north central Colorado. Mule deer were the primary species affected and accounted for 41 of the 49 cases, but six Rocky Mountain elk and two white-tailed deer were also affected. Within the free-ranging cervids, pre clinical prevalence (based on the detection of PrP^{CWD} by IHC) is higher in mule deer (<1-15%) than elk (<1%) (Miller *et al*., 2000). Overall, in free-ranging deer in the historic enzootic area's in Colorado/Wyoming, prevalence among hunter killed animals is <10% (Williams and Miller, 2002). During the 2002 hunting season a rapid screening test (BioRad ELISA) was applied on a volunteer basis to screen more than 25,000 specimens from Colorado harvested elk and deer. The IHC was used as a confirmatory test for those samples indicated positive screen test results and findings from this survey will be available soon.

3.4.1.2 Chronology of distribution in CANADA

CWD is thought to have entered Canada with elk imported from the United States in the late 1980s. Investigations have shown that a farm (later referred to as the 'source farm') established in Saskatchewan in 1987 spread infection via elk sales to 21 other farms, which in turn infected 17 more farms in the same province. The source farm held 63 elk imported from the United States, including from at least one farm that was subsequently confirmed as CWD-infected. The Canadian elk herd was expanding rapidly in the late 1980s and foundation herds that had imported elk from the United States traded animals throughout Saskatchewan and Alberta. At this time, Manitoba and British

Columbia did not allow elk farming and, from 1988, Provincial authorities prohibited the entry of cervids into Alberta.

Due to concern about the entry of bovine tuberculosis, the Canadian government prohibited the import of cervids from 1990 to 1999. From 1999, cervids could again be imported from the United States, under conditions designed to prevent the introduction of CWD. Some Provinces conducted testing for CWD but there was no official control program for CWD until 2000. In 2001, CWD was made reportable at the federal level. Movement of live elk was key to the spread of CWD in Saskatchewan. According to the Canadian Food Inspection Agency's investigations, the source farm sold 864 elk in the 1990s. A total of 274 elk that had been sold within 36 months of quarantine being imposed in 2000 were traced, slaughtered and tested to reveal 21 CWD-infected animals (prevalence 8%). Depopulation and testing of the remaining 403 animals in the source herd revealed a within-herd prevalence of 11%. The CFIA investigated all herds that had received cervids from the source herd. The last reported case of CWD in farmed elk was in March 2002.

As said earlier, CWD in a mule deer in Canada was first reported at the Toronto Zoo in Ontario in 1976.

In summary, since 1996, CWD has been diagnosed in 42 elk farms and one white-tailed deer farm in Canada (Saskatchewan and Alberta). 95% Of infected elk herds had only a few (1-3) infected animals as diagnosed by IHC on the brain and most (91%) elk diagnosed with CWD were at a pre-clinical stage. Approximately 65% of infected herds in Saskatchewan had a prevalence of infection less than 5%. While animals less than 12 months of age have been diagnosed with pre-clinical infection by IHC, the youngest elk diagnosed with clinical CWD was 17 months old. With elk, as with deer, animals of all ages and both sexes have been found infected with CWD and no bias has been evident.

3.4.2 Evidence for lateral transmission

There is considerable evidence that CWD is both infectious and contagious but specific details of its transmission remain to be determined. However, historically the epidemiology of CWD is not supported by evidence of being a feed-borne disease like BSE, associated with rendered ruminant meat and bone meal (MBM). Evidence for this

includes (1) the observations that captive cervids without records of being fed with animal-protein also succumbed to the disease and (2) free-ranging animals are unlikely to have access to compound feed stuffs.

Epidemiological studies strongly suggest that lateral transmission similar to that experienced in scrapie epidemics, occur in CWD and is the most important factor with impact on the spread of the disease (Williams and Young, 1992, Miller at al., 1998, 2000). Indirect transmission via environmental contamination may play a role in the natural dynamics and persistence of CWD (Miller *et al,* 2000). The CWD agent has been demonstrated in lymphoid tissues of the alimentary tract that suggests that the agent may be shed through the alimentary tract (faeces and saliva). Contaminated pastures used by captive cervids appear to have served as sources of infection in some CWD epizootics. This apparent persistence of the CWD agent in contaminated environments might represent a significant obstacle to eradication of the disease from either farmed or free-ranging cervid populations. The potential role of invertebrate and/or vertebrate reservoirs in CWD epidemiology warrants further study, as does the influence of climate on disease persistence, especially in free-ranging populations. Rapid increases in prevalence within captive herds suggest transmission may be quite efficient, at least at a local level. In Saskatchewan, 4 elk farms have not been allowed to restock with grazing, food-producing animals unless they first stock sentinel cervids that would be slaughtered and tested for CWD after 4 years on the farm(s). If the sentinels test negative for CWD, the Canadian Food Inspection Agency would then lift quarantine and remove any restrictions on farm use.

There is less evidence for the existence of maternal transmission but because this cannot be distinguished from the high component of lateral transmission, it is not possible to exclude it. Placentomes, ovaries and fetal tissues from two mule deer in term pregnancy were examined with IHC and PrP^{CWD} was not detected (Spraker et al. 2002a) unlike the finding of PrP^{Sc} in pregnant domestic sheep (Tuo *et al.* 2002). Tuo *et al*., (2002) demonstrated that accumulation of PrP^{Sc} in uterine-placental epithelial cells in the placentome was determined by the pregnancy status of scrapie-infected ewes. The distribution of PrP^{Sc} plaques in placentomes showed a tendency toward increased size and number of placentomal PrP^{Sc} plaques from the endometrial stalk (maternal side) to

chorionic plate (fetal side). Maternal transmission alone is unlikely to sustain epidemics of CWD (Miller, 2002).

Both sexes and a wide range of age classes of animals can be affected, underscoring the likely importance of animal-to-animal (lateral) transmission in sustaining epidemics. Both intra- and inter-specific transmission (e.g., mule deer/white-tailed deer, elk/white-tailed deer) probably occurs. It is not known when during the course of the disease an animal becomes infectious but it appears likely that PrP^{CWD} shedding is progressive through the disease course. The presence of PrP^{CWD} early in the incubation time in alimentary tract associated lymphoid tissues suggest that shedding may start early (Sigurdson et al., 1999).

In summary, lateral transmission, compounded by animal movements is the most important factor in the spread of CWD. Indirect transmission via environmental contamination may play a role in natural dynamics and persistence of the disease and thus exacerbates epidemics and may present an obstacle to eradicating CWD from infected premises. This possibility and other epidemiological uncertainties also present significant obstacles to eradicating CWD from wildlife.

3.4.3 The origin of CWD

The origins of CWD are unclear. There is no epidemiological evidence that would suggest a possible origin of CWD. As indicated above, there is also no evidence to support a feed-borne common source origin of CWD. Other hypotheses as to the origin of the disease might include the following:

1. A low level of endemic disease present in susceptible wild cervids, possibly confined to the Western USA, before they were brought into captivity. The resultant closer contact between animals in captivity then increased the frequency of lateral transmission of the disease.
2. Infection of deer by a strain of scrapie that has adapted to cervids (Williams and Miller, 2002).
3. A genetic form of TSE arising in deer, with subsequent natural transmission or a spontaneous conformational change of the prion protein occurring in mule deer, with

subsequent transmission to other deer and to elk. With either possibility, the critical genetic event could have arisen in the wild or in captive stock.

4. Exposure to a currently unknown TSE; expressing the possibility, borne particularly out of the infancy of the study of diseases of wildlife, that there could be undetected TSE or prion diseases in other species.

None of these hypotheses provide particularly plausible explanation but further consideration of the evidence against a sheep scrapie origin is necessary as this has long been a known reservoir of a TSE in domestic animals. Given the enzootic occurrence of scrapie in the NA, a scrapie origin might be considered the favoured theory, but there are substantial counter arguments. Scrapie in sheep has an almost world wide distribution and is present in many countries that harbour free-ranging deer but CWD has not been reported in deer populations of countries outside of NA. Although CWD transmits to goat (Williams and Young, 1992) and to sheep (Hamir *et al*., 2003) by IC inoculation, the incubation periods (> 6 years in goats) produced suggest a large species barrier and this is not what might be expected if the agent were originally a sheep scrapie agent strain. Also, biological strain-typing in inbred mouse strains has shown that the CWD agent differs from the BSE agent and from strains of scrapie tested thus far (Bruce et al., 2000). Lastly, comparisons of abnormal PrP glycoform patterns from CWD affected deer and elk, scrapie affected sheep and cattle, did not provide reliable indications of TSE infections of common origin among the species studied (Race et al 2002). There is therefore, no evidence that it has arisen from the other TSEs such as scrapie, BSE or CJD, consistent with evidence from strain typing studies, but the possibility cannot be ruled out entirely.

3.5 PATHOGENESIS

There are no reported studies of tissue infectivity bioassays in CWD because there are currently no adequate biological models available to detect CWD infectivity and because the substantial resources necessary to conduct bioassays in deer and elk have not

been allocated. This is an important omission in the research, which prevents any quantification of infection relative to tissue/organ.

3.5.1 Distribution of lesions / PrPCWD in clinically affected animals

Relatively minor differences in the distribution of lesions and PrP^{CWD} have been reported among the three affected species. As in other TSE the most striking changes are seen in the central nervous system. Spongiform changes are present in the medulla oblongata, especially the parasympathetic vagal nucleus and in the thalamus, hypothalamus and olfactory cortex and are often severe. PrP^{CWD} as demonstrated by IHC is found in the brain, palatine tonsils, visceral and regional lymph nodes, Peyers patches and other lymphoid tissue of small and large intestine and spleen of affected deer (Sigurdson et al., 1999). In the brain, the disease specific PrP accumulation and spongiform change is seen initially in the dorsal motor nucleus of the vagus nerve (Williams and Young, 1993; Williams and Miller, 2000). Sigurdson *et al* (2001) detected PrP^{CWD} in the brain stem, spinal cord, , pituitary (*pars intermedia and pars nervosa)*, vagosympathetic trunk, sympathetic trunk, nodose ganglion, myenteric plexus, adrenal medulla, pancreatic islets, brachial plexus, sciatic nerve, **but not** in the trigeminal (gasserian) ganglion, coeliac ganglion, cranial cervical ganglion or spinal nerve roots (dorsal and ventral) in a small sample (n=2-6) of mule deer naturally affected with CWD. These findings suggest that there is, at least in the clinical disease, extensive involvement of multiple organ systems, including central and peripheral nervous tissues, endocrine organs and alimentary tract, the last suggesting a possible means of agent shedding. Immunohistochemical evidence of disease specific PrP has not been found in the mucosa of the abomasum and intestines, thymus, bone marrow, skeletal muscle, liver, lungs, myocardium, walls of vessels, kidney, bladder, ovary, endometrium, testis, epididymis, sebaceous and sweat glands, and epidermis of skin of affected deer.

Brain lesions associated with clinical disease in **deer** have been found by 16 months and in elk as from the age of 12 months, whereas immunohistochemical demonstration of PrP^{CWD} is achieved much earlier, sometimes several months, or up to a year, in both lymphoid tissues and CNS.

Diagnosis based solely on IHC examination for PrP^{CWD} in tonsils and retropharyngeal lymph nodes is less reliable in elk than in deer (Balachandran, pers.com.2003).

3.5.2 Studies of PrPCWD in pre-clinically infected animals.

3.5.2.1 Studies of PrPCWD in naturally exposed animals

Histopathological examination and PrP-IHC staining was carried out on at risk, clinically normal elk population from the 'source farm' in Saskatchewan (Canada) and of 398 elk examined, 38 were positive by both test criteria and a further 8 were positive by PrP-IHC without histological changes (Balachandran, 2003).

Genetic analysis of the open reading frame of the cervid PrP gene was done on 32 of the 46 test positive elk; 31/ 32 were homozygous for 132M. The single heterozygous (LM) elk was a 4 years old with moderate spongiform changes and IHC staining in the DMNV. In all CWD affected elk disease-specific PrP^{Sc} was identified in the DMNV which is postulated to be the primary site of entry of the CWD agent into the brain. Eight elk had small, focal deposits in the ventromedial border of the DMNV in the absence of clinical disease or histopathological lesions of CWD. Eleven elk had a very mild and focal spongiform change confined to the DMNV but demonstrated conspicuous PrP^{Sc} deposits at the same site. These findings suggest that variable levels of PrP-Sc accumulation appear to precede spongiform lesions in the incubation period. Ages of CWD positive animals ranged from 1-13 years; there was no correlation between the severity of the lesions or extent of IHC positive deposits and the age of the animal at euthanasia. One test of deer antler velvet collected from an elk 3 months before the animal developed clinical CWD showed no detectable PrP^{CWD} by routine immunoblotting and IHC (Balachandran, pers.com.2003).

3.5.2.2 Studies of PrPCWD in experimentally infected animals

The pathogenesis of CWD has also been studied in experimentally infected mule deer via oral exposure to brain homogenate from clinical case of CWD (Sigurdson *et al*, 1999). PrP^{CWD} was detected in alimentary-tract-associated lymphoid tissues (one or more

of the following: retropharyngeal lymph node, tonsil, Peyer's patches and ileocaecal lymph node) as early as 42 days p.i. and in all fawns examined thereafter (53 through to 80 days p.i when the study was terminated). No PrP^{CWD} was detectable in neural tissue in any fawn. In ongoing pathogenesis studies (Williams et al., unpublished) in deer brain, PrP^{CWD} has been found at the age of 5-6 months when it was also found in lymphoid tissues.

3.5.3 Conclusions

In deer and elk, PrP^{CWD} has a very wide and early tissue distribution, which resembles the distribution of scrapie and BSE agents in tissues in TSE-susceptible sheep and is different to that seen in BSE in cattle. However, tissue distribution is not identical for deer[4] and elk. In the latter species it accumulates later in the incubation period into detectable levels. This widespread distribution of PrP^{CWD} early in the incubation period presents significant, if not insurmountable, difficulty with respect to the potential for decisions on the removal of specified risk materials (SRM) in CWD.

3.6 DIAGNOSIS

3.6.1 Clinical diagnosis of CWD

Clinical signs of CWD are not specific. A consistent clinical sign of CWD in deer and elk is progressive weight loss. Behavioural changes also occur in the majority of cases, including decreased interactions with other animals, listlessness, lowering of the head, drooping ears, blank facial expression and repetitive walking in set patterns. In elk, behavioural changes may also include hyper-excitability, nervousness, ataxia and head banging. Free ranging CWD affected elk may loose fear of humans. Affected animals

[4] Tables in annexe 1 provide a summary overview of PrP^{CWD} distribution in mule deer only. This should be viewed with some caution with regard to comparisons with similar data on risk tissues based on the results of infectivity assays (in mice and, for BSE, in cattle) in other species with TSEs. The overview given in the table is based on immunohistochemical detection of PrP, the sensitivity of which in peripheral tissues of deer relative to infectivity assays has not been determined. However, an immunoassay (CDI) shown to be capable of detecting PrP^{BSE} in the brainstems of cattle with a sensitivity similar to that of the infectivity levels determined by end-point titration in Tg(BoPrP) mice and capable of detecting PrP^{CWD} , offers prospects for sensitive detection of the range of risk tissues in deer/elk (Safar *et al*. 2002).

continue to eat grain but may show decreased interest in hay. In deer and elk polydipsia and polyuria also commonly occur. Excessive salivation and grinding of the teeth are also observed. The clinical disease is progressive and always fatal.

In captive herds, experiencing a new outbreak of CWD, animals frequently have a history that includes sporadic cases of prime aged animals losing condition, being unresponsive to symptomatic treatment and dying from pneumonia. This aspiration pneumonia, presumably caused by difficulty in swallowing and by ptyalism, may lead to misdiagnosis of the condition if there is not histological and/or immunohistochemical examination of nervous or/and lymphoid tissues. "Sudden deaths" following handling also have been reported as the index cases in some situations as have unusual traumatic losses.

Most cases of CWD occur in adult animals. The majority of CWD-affected animals are 3-5 years of age. The oldest elk animal with CWD was >15 years old. The clinical course of CWD varies from a few days to approximately a year, with most of animals surviving from a few weeks to three or four months. Caretakers familiar with individual animals often recognise subtle changes in behaviour well before serious weight loss occurs.

Differential diagnosis should be made with other diseases including mineral deficiencies leading to neurological signs in deer and elk e.g. fading elk syndrome, listeriosis, copper deficiency,…etc.

The **incubation period** range in naturally occurring CWD is not known. Evidence of CWD infection (not clinical disease) has been seen in deer fawns and elk calves by about 6 months of age (Dr. Spraker). The youngest **naturally infected mule deer** diagnosed with clinical disease was 17 month of age suggesting 16 to 17 months as an approximate minimum incubation. CWD has been diagnosed in a 24 months old Rocky Mountain elk (Ball, 2002).

Clinical disease in **experimentally infected elk** was observed by 12 months (range 12-34 months) post-exposure. In experimentally infected deer, minimum incubation was approximately 15 months and mean time from oral infection to death was approximately 23 months (20->25 months).

3.6.2 Laboratory Diagnosis

Post mortem diagnosis

Gross lesions seen at necropsy reflect the clinical signs, primarily emaciation. Aspiration pneumonia, which may be the actual cause of death, is also a common post-mortem finding in animals affected with CWD.

On microscopic examination, spongiform lesions of CWD in the central nervous system resemble those of other TSE's. Lesions are usually found in several nuclei in the medulla oblongata, pons, mesencephalon and telencephalon in clinically affected animals (Williams and Young, 1993; Spraker *et al*., 1997). The parasympathetic vagal nucleus in the dorsal portion of the medulla oblongata at the obex is the most important site to be examined for diagnosis of CWD especially in apparently clinically normal animals (Peters et al, 2000; Spraker et al., 2002).

Immunostaining of tissues using PrP antibodies can demonstrate disease specific prion protein in the brain, palatine tonsils, visceral and regional lymph nodes, Peyers patches of the small intestine, lymphoid tissue of the large intestine and the spleen of affected deer. Immunohistochemistry (IHC) currently used as the 'gold standard' in testing for different TSE's, is also used to test brain tissue for the presence and accumulation of PrP^{CWD}, the protein marker to diagnose CWD. The area of the brain used for testing (parasympathetic vagal nucleus of the medulla at the obex) is critical and if the correct area of the brain is not tested, this fact should be included in the report. Testing of both brain and lymphoid tissue is preferred.

The current rapid tests used for screening for BSE in Europe are being evaluated for their usefulness as screening tests for CWD. The Bio-Rad CWD ELISA test used on lymph node has recently been licensed in the US for mule deer, elk and white-tailed deer.

Immunohistochemistry (IHC) and Bio-Rad ELISA both provide reliable results in testing for CWD. The latter test was used in some veterinary diagnostic laboratories on collected samples (Colorado and Wyoming). In comparing the Bio-Rad with using IHC, the Bio-Rad was extremely accurate with a sensitivity of 98-99%. All animals with positive results on the ELISA test are confirmed by the IHC-test.

To date slightly over 27,000 tests in 25,000 animals with approximately 200 positive animals (mule deer, elk and white-tailed deer) have been run using the Bio Rad Elisa for free-ranging cervid surveillance.

In regard to PrP^{CWD} detection in the spleen, a study of 26 mule deer (16 free-ranging and 10 captive) that were in terminal stages of CWD, found PrP^{CWD} in the spleen of 53% and 44% of the free-ranging and captive deer respectively(Spraker 2002a).

3.6.3 Laboratory diagnosis in live animals

Mule deer and white-tailed deer

Tonsillar biopsies have been assessed for the diagnosis of CWD in live animals (Wild *et al*, 2002; Wolfe et al., 2002). This technique is useful for the preclinical diagnosis of CWD in farmed live mule deer and white-tailed deer. PrP^{CWD} accumulates in tonsillar and lympoid tissues in an early stage of the infection and can be detected with IHC from 2 to 20 months before CWD-related death and up to 14 months before onset of clinical signs of CWD. These studies suggest that tonsillar biopsy is a valid method for detecting CWD in live deer during incubation stage, and may be used as a ante-mortem and pre-clinical diagnosis and as an adjunct management tool. This technique as a practical management tool under field conditions (i.e involving the capture, anaesthetic and biopsy of wild deer) is currently been evaluated (Wolfe et al., 2002).

Elk

Because PrP^{CWD} does not appear to accumulate in lymphoid tissues to the same degree in elk as occurs in deer, tonsillar biopsy is not a currently applicable technique for elk (Spraker, unpublished data, in Wild *et al*, 2002).

A third eyelid test as has been used in sheep for the diagnosis of scrapie was examined for the pre clinical identification of infected animals (O'Rourke *et al.,* 2002), however, this approach does not seem feasible in deer and elk because of the very limited amount of lymphoid tissue associated with the third eyelid in these species (Miller and Spraker unpublished data).

3.7 SURVEILLANCE

Presently, two surveillance strategies are generally used:

1. Passive surveillance: testing of sick or 'target' animals discovered by or reported to wildlife agencies. 'target' animals are defined as deer or elk of 18 months of age or older that are emaciated and showing some signs including abnormal behaviour, **increased** salivation, tremors stumbling, in-coordination, difficult swallowing, excessive thirst and/or urination.
2. Active surveillance: conducting 'at random' surveys of normal deer and elk killed by hunters or agency personnel.

It should be noted that introduction or more general use of rapid-tests would facilitate the surveillance of CWD. As indicated earlier, results produced by screening test should be confirmed by the 'gold standard' test (IHC).

3.7.1 Type and organisation of surveillance in free-ranging cervids

3.7.1.1 USA

A series of surveys for CWD in free-ranging animals has been ongoing since 1983. Three types of surveillance are undertaken :

- Targeted surveillance is the collection of any cervid that exhibits clinical signs of CWD.
- Hunter harvest surveillance is the collection of the heads of hunter-harvested cervids to test for CWD. Also included are samples collected from road-killed animals.
- Outbreak surveillance is the collection of specified numbers of animals to determine the rate of infection and the extent of the infected area identified through either targeted, hunter harvest surveillance or animals killed by wildlife management agencies.

Surveillance of free-ranging cervids for CWD has been ongoing in Colorado and Wyoming since the late 1970s (first cases were found in 1981). CWD-positive free-ranging animals have now also been found outside the historic enzootic area. In

Wisconsin, over 30 CWD positive deer have been identified from samples collected during the 2002 gun deer season (2001, 3 positive cases). Further cases are to be expected as the hunting season proceeds. A free-ranging white-tailed deer was just recently (November 2002) found in northern Illinois. In June 2002, one positive animal was found in New Mexico (a mule deer collected from the White Sands Missile Range) and more CWD positive animals were identified during the 2002 hunting season.

In summary, CWD has been found in free-ranging mule deer in Wyoming, Colorado, Nebraska, South Dakota and New Mexico. It has been found in free-ranging elk in Wyoming, Colorado, and South Dakota. It has been found in free-ranging white-tailed deer in Wyoming, Colorado, South Dakota, Nebraska, Wisconsin, and Illinois. With continuing and planned levels of surveillance this may change over a period of only a few months.

3.7.1.2 Canada

Since April 2001, CWD has been reported in 7 wild deer in Saskatchewan as a result of surveillance in free-living deer. A total of 16,850 wild cervids in Canada, were tested in the period 1996-2002, including 11,205 in Saskatchewan, 2,892 in Alberta and 2,407 in Manitoba. Provincial Government surveillance is focused on areas in which infected herds or infected wild cervids have been found and on the borders between provinces. However, most testing is conducted at random. The samples are collected from cervids shot by provincially licensed hunters and from animals killed on the roads.

3.7.1.3 Planned surveillance on free ranging Cervidae in NA

It is difficult to specify how much surveillance will be carried out in free-ranging cervids in North America in 2003 as this depends on differences between states (USA) and provinces (Canada) and the type of cervids targeted (farmed/free-ranging). Many states have plans to test hunter harvested animals this year and the number of samples to be tested has been estimated to be 50,000 to 200,000. There has been an increase in

numbers tested each year indicating an increasing level of co-operation from the industry and state regulatory officials as they became more aware of the problem.
Background information on the populations of cervids in different areas and on numbers tested and cases confirmed in relation to different types of management is essential in further determining the strategies for control and/or eradication

3.7.2 Surveillance in farmed cervids

CWD has been diagnosed in farmed elk herds in a number of States in the USA and in two Canadian provinces.

3.7.2.1 USA

The national surveillance plan for farmed cervid herds includes

- mandatory death reporting
- CWD testing of all animals, except calves, which are slaughtered or die on the affected premises.
- Individual animal identification and annual census.

Surveillance for CWD in farmed elk began in 1996 and has been a co-operative effort involving State agriculture and wildlife agencies and the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). Farmed cervid surveillance has been increasing each year since 1997 and will be an integral part of the USDA program to eliminate CWD from farmed elk.

The farmed cervid surveillance program and the surveillance program for wildlife are interdependent. Particular aspects of surveillance programmes depend upon circumstances in each State. For areas with known CWD infections, estimates of disease prevalence can be used to judge the effectiveness of management actions and to evaluate disease dynamics in the context of ecological research questions. Surveillance activities are also needed to satisfy public and management information needs.

CWD positive herds in the United States include South Dakota, Nebraska, Colorado, Oklahoma, Kansas, Montana and Minnesota. Also CWD has been diagnosed in farmed white-tailed deer in Wisconsin.

3.7.2.2 Canada

While there was no active Federal surveillance for CWD until 2000, Provincial Governments have conducted some surveillance of farmed cervids for CWD since 1997. Provincial programs (to 31-12-02) have detected 4 CWD-infected farms (2 elk farms in Saskatchewan, 1 elk farm in Alberta and 1 white tailed deer farm in Alberta) for a total of 11,025 animals tested (6,482 and 4543 respectively). In Manitoba, 72 farmed cervids have been tested negative by IHC since 2001. Since 2002, the provinces of Saskatchewan, Alberta and Manitoba have required CWD testing of all adult, farmed cervids that die on farm or are slaughtered.

Since the eradication program commenced in February 2000, the CFIA has slaughtered approximately 8,300 farmed elk on affected farms (40 in Saskatchewan and 1 in Alberta) and tested 7,153 adult animals (99 % elk) to detect a total of 231 elk infected with CWD. The infected white tailed deer farm in Alberta was destroyed in 2003 and testing revealed that only one cervid was infected with CWD. The CFIA is in the process of conducting retrospective inspections of farms that have imported from the United States, with emphasis on those where imported animals died within 3 years of importation.

A voluntary national CWD certification program was recently introduced to provide access to herd replacements of known ('certified') CWD status and to meet the requirements of trading partners. Subject to conditions, herds that have been enrolled in provincial CWD certification programs can enter the national program at higher than entry level status

3.8 CONTROL STRATEGIES

3.8.1 In the USA

There are five general reasons why people own elk and deer in the US. The four primary reasons are purely commercial endeavours and include harvest of antlers for the Korean market, selling trophy hunts, selling of meat, and selling breeding stock. The fifth reason is purely for pleasure i.e. the deer or elk are kept as pets and are not used commercially.

Control measures in general include prevention of introduction, notification of the disease, control or ban on movements, quarantine, eradication of affected herds, compensation and measures to prevent/stop spread from free range to farmed (or vice versa). Because of the commercial aspect of game ranching, animals were commonly moved across the US and Canada. Recently, laws have been made to prevent the movement of these captive animals across state lines. Some states will not allow any parts of animals into their state if the origin of the meat/tissue is from area the CWD is known to occur.

There is also some natural movement of deer and elk across state lines. Knowledge of herd management, prevalence of CWD and susceptibility factors may provide additional support for efficient controls: e.g. a hierarchy of prevalence is likely among the species (white tailed deer >mule deer>elk) given that white tailed deer are more social and found at higher densities.

Several **USA states** that have recently banned or restricted the importation of deer species including North Carolina, Michigan, Vermont, Tennessee, Texas,(March 2002) Nebraska, Wisconsin, New York, Colorado and Arizona. Upon recognition of the disease in New Mexico in free-ranging mule deer, the state immediately stopped any importation of deer or elk. Following screening of herds, herd certification may be an option. However, given limited knowledge on the incubation of the disease and its variation in clinical presentation it is likely to take a period as long as 5 years of surveillance of all juvenile and adult mortality before a farmed herd may be certified free of CWD.
The US FDA Center for Veterinary Medicine announced in November 2002 a proposed (still under discussion) policy on rendering tissues from cervids from CWD positive areas or herds.

3.8.2 In Canada

Key elements of the Canadian eradication for farmed and captive cervids are as follows

- CWD is reportable under the federal Health of Animals Act (since 2001).
- The finding of an infected animal (confirmed by IHC in a CFIA laboratory) triggers a series of events :
    1. Imposition of quarantine on all animals and animal products at the affected farm.
    2. Slaughter of all cervids.
    3. Testing of all adult cervids in a CFIA laboratory.

The CFIA traces all animals that have entered the farm and traceout animals that have left the farm up to 60 months before the date of diagnosis or the date the disease was introduced to the farm. Animals that have left the farm within 36 months of diagnosis are slaughtered and tested while those that departed more than 36 months but less than 60 months before diagnosis are the subject of movement control and regular CFIA veterinary inspection for the remainder of the 60 month period. At the end of this time, if the animals are clinically normal, the CFIA lifts all restrictions. All cervid species are considered to be susceptible to CWD. All animals in an infected herd and all animal products are considered at risk of CWD contamination. The animals are humanely destroyed then carcasses are disposed of by incineration or deep burial. Products (antler velvet, semen, embryos) from tested CWD positive animals are destroyed. Siting of burial pits requires approval from environmental protection authorities. Pits are located in remote rural areas and subject to ongoing monitoring. The site should not be disturbed for several years.

All herd owners are required to cleanse and disinfect farms before re-population is permitted. This involves cleaning and washing equipment, vehicles and surfaces with hot water and detergent, then sanitising with a 2% solution of sodium hypochlorite or sodium hydroxide (1N). Wooden structures that cannot be decontaminated must be removed. Soil and manure must be removed from heavily used areas, such as gates and lane ways (i.e. elk ‘runs’), and areas of pastures where animals may have congregated, to a depth of 5 cm below the level of soil disturbance by hooves. This area should be re-covered with soil or gravel to a depth of 10 cm. All contaminated soil, materials, feed and ash resulting

from incineration must be buried in a site selected to have negligible environmental impact

The CFIA conducts an epidemiological investigation to determine what further action must be taken. Key factors include: the number of animals infected, the extent to which disease has been manifested clinically and the estimated duration of infection. Indirect evidence (e.g. a history of animals dying under circumstances that suggest the presence of CWD) is also taken into account. All restocked herds must participate in a health certification program, with a requirement for CWD testing of all adult cervids that die. (Canadian Food Inspection Agency (CFIA) Manual of Procedures, Chronic Wasting Disease, July 2002)

Since the eradication program commenced in February 2000, the CFIA has slaughtered approximately 8,300 farmed elk on affected farms (40 in Saskatchewan and 1 in Alberta) and tested 7,153 adult animals (99 % elk) to detect a total of 230 elk infected with CWD. The infected white tailed deer farm in Alberta was destroyed in 2003 and testing revealed that only one cervid was infected with CWD. It has cost the federal government CAD 33 million to compensate the farmers. The CFIA has allowed all but 4 farms to restock with cervids after completion of cleaning and disinfecting. The four farms have been placed in long term quarantine and will be required to implement a sentinel program for at least 4 years before the CFIA will allow restocking with food producing livestock species that graze. (B. Peart, personal communication, 2002).

3.8.3 Economic impact

It is obvious that there has been significant impact on the NA farmed cervid industry but the total effect is difficult to quantify. There has been some impact on sale of hunting licenses in different US states (e.g.Wisconsin). Public awareness has been raised by multiple forms of outreach by many agencies. As indicated before, a huge cost is involved in the compensation of Canadian farmers where animals were eradicated on CWD positive farms. The cost of quarantaine of farm and grassland in an attempt to reduce the environmental contamination following CWD in a farmed herd is difficult to quantify. CWD has also had a major impact on the deer and elk farming industry. In Canada, elk are raised for the production of antler velvet and meat and for trophy/hunting. About 70% of velvet antler was formerly exported to South Korea. In the

course of Canadian eradication activities and the detection of an increasing number of cases in 2000-2001, some trading partners closed their markets to Canadian cervids and cervid products, including semen, embryos and velvet. It is difficult to quantify the total economic impact of this market closure. The price per pound of unprocessed velvet dropped from around Can $60 in 2000 to Can $22 in 2002 (R. Nixdorf 2002). Average prices paid for all categories of elk in Saskatchewan were at their peak between 1995 and 1997, ranging from Can $9,500 to Can $24,000. The average price decreased dramatically in 1997-2000 due primarily to the financial crisis in Asia. The average price continued to decrease between 2000 and 2002 due to the introduction of CWD, the loss of market opportunities and the loss of confidence in the industry. (A.Dagenais, Pers. Com.).

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4 TSE'S IN CERVIDS IN EUROPE

4.1 THE HISTORICAL AND CURRENT SITUATION IN GREAT BRITAIN IN RELATION TO BSE

There is epidemiological and biological strain typing evidence that the occurrence of spongiform encephalopathies in closely related wild ungulate species held in British zoological collections contemporaneously with the epidemic of BSE, were due to food borne exposure to the BSE agent via contaminated proprietary ruminant feedstuffs. Such cases occurred only in species within the family Bovidae (subfamilies *bovinae* and *hippotraginae*) (Kirkwood and Cunningham, 1994) and a considerably greater range of species, not only within the order *Artiodactyla*, but across several other orders, was exposed to feeds containing animal proteins. Within the Artiodactyla, an estimated 62 species were held in British zoos in 1989 (Kirkwood and Cunningham, 1994) and undoubtedly this included members of the family *Cervidae*. The extent to which such species were exposed to commercial feedstuffs or supplements at the time is not known, but the practice was commonplace.

4.2 PAST AND CURRENT SURVEILLANCE IN EUROPE

There is no published information on the possible occurrence or surveillance for TSE's in cervid species on the European continent. Throughout the world (but particularly in Europe, NA and Australia) pathological examinations will have been carried out on numerous species of deer that have died in, or have been culled from zoological collections and in many cases this will have included histopathological examination of the brain. However, there are no correlated analyses of the numbers involved or the resulting pathological reports.

Several zoological gardens and wildlife research institutes were contacted for further information on surveillance of cervidae and from data received it is concluded that currently minor surveillance activity is on-going or planned on CWD in cervidae.

In 1991 it was proposed in **England** that the brains from a sample of farmed red deer would be examined for evidence of spongiform encephalopathy (SE). Given the estimated population and in order to demonstrate a potential prevalence of 0.1% with 99% certainty, there was a requirement for a sample of approximately 4,600 brains. Because of pressures on resources at the time this investigation was not pursued. Prior to and over the course of the BSE epidemic in Britain small numbers of deer brains have been examined by routine diagnostic histopathology with no evidence of an SE. Current surveillance of deer for TSE in Britain has accumulated (in 2002) samples from more than 300 deer, to include brain, lymph nodes, spleen and intestine (S. J. Ryder, personal communication). The material has been collected from wild deer and the species represented include mainly roe (*Capreolus capreolus*), sika (*Cervus nippon*), fallow (*Dama dama*), red (*Cervus elaphus*) and muntjac (*Muntiacus reevesi)* deer. Immunohistochemical studies of the tissues will be based on methods used to diagnose CWD in NA. An analysis of the deer industry in Britain has been conducted to form the basis for further structured surveillance of deer species. Experimental studies of the transmissibility of BSE to red deer have been proposed in conjunction with US collaborators. These studies also propose to apply pathological examinations to

phenotype any SE disease produced in the deer using immunohistochemical and western blotting methods. Comparisons of any resulting phenotype characterisation will be made with cases of CWD in NA deer species and with cases of scrapie in domestic sheep.

Substantial efforts are being undertaken in **Germany** (collaboration between 'Forschungsverbund Berlin' (IZW), CENAS AG, Kulmbach and the Federal Research Centre for Virus Diseases of Animals) and funded (summer 2002) by the German Federal Ministry of Education and Research (BMBF). A testing procedure for CWD will be set up which allows the screening of a larger number of wild ruminants in regards of TSE's. A total of 10,000 wild ruminants older then 18 months (captive, fallen and hunted animals) will be screened for TSE's (CWD, BSE, Scrapie). Immunochemical, histological and immunohistochemical methods will be employed. For primary screening the BioRad Assay will be used; for validation samples from deer which have been positively diagnosed for CWD will be obtained from collaborating laboratories in Wyoming. So far (January 2003) samples of ca. 500 heads of animals have been collected during the year of 2002. All animals have been hunted and derive from different regions all over Germany with a high occurrence of BSE and Scrapie. Prior to hunting, no characterisation of the animals in respect to symptoms of a putative CWD disease has been performed. However as the result of the ongoing collaboration between hunters and the IZW future collections of samples will take this consideration into account. Due to lack of approval by the owners, no samples from captive animals have yet been taken.

In **Norway,** at the National Veterinary Institute Section for Wildlife Diseases, a few individual cervids have been tested (three moose, one red deer) with an ELISA for PrP^{sc} (Bio-Rad). During 2001/2002, the brains of a total of 77 moose, 15 red deer and 14 roe deer have been subjected to histopathological examination and there has been no sign of spongioform encephalopathies in any of the material tested. Plans for research within the area of TSE / CWD in cervids : suitable material from cervids for autopsy will be tested by the ELISA method and histopathology of the appropriate area of CNS. Hunters in the area close to the laboratory are asked to supply with heads from game shot during ordinary hunting.

Sweden has no official surveillance programme in wild ruminants to look for CWD in cervidae, but the Department of Wildlife Diseases (DWD) has been working on a scheme to look into the problem. The DWD is a pathology based dep and do autopsy on fallen wildlife which are sent to the National Veterinary Institute and all expenses paid by the Institute (i.e.the money is actually coming from a hunter administrative fund). This is quite an effective system and the hunters are active and well aware of the good use of disease monitoring through post mortem exminations. The DWD has been collecting brain material so far but this has not been submitted to be analysed. The Biorad ELISA used for cattle and sheep will be used on cervids as well.
Research and funding: last year, the National Veterinary Laboratories from three Nordic countries, Finland, Sweden and Norway, applied to the Nordic Joining Committee for Agricultural Research, for funding. The research project aims at the testing of 600 moose (200 from each country) for TSE, and to investigate whether a disease condition characterised by wasting and neurological signs (relatively common in Sweden).
The project was not granted funding and was not forwarded for application again.

In **Switzerland**, a surveillance programme for farmed cervids older than 2 years has started in January 2003 and is financed by the Swiss Federal Veterinary Office. The brain, lymph nodes and tonsils of fallen stock and slaughtered animals are collected and examined by histology and immunohistochemistry at the Swiss Reference Laboratory for spongiform encephalopathies in animals. The aim of the study is to screen at least 200 cervids for TSE's and other neurological diseases. The majority of the farmed cervids in Switzerland are fallow deer (*Cervus dama*).

5 POSSIBLE GLOBAL OCCURRENCE OF TSEs IN FARMED CERVIDAE

A case of CWD occurred in an elk imported into Korea from Canada (Sohn et al., 2002). New Zealand has potentially also been exposed to the risk of CWD through the importation of elk from Saskatchewan, although none of the animals imported came from

herds subsequently found infected. During the 1990s, New Zealand imported elk semen and embryos from a herd in Saskatchewan in which CWD was diagnosed in 2001.

Between 1995 and 1999, 4 animals died in the export herd under circumstances that could have been associated with CWD. Exports of embryos occurred in 1990 and exports of semen in 1989, 1994, 1996 and 1997. The CFIA tested all adult animals in the herd and did not identify any infected animal from which semen had been exported. From epidemiological investigations, the CFIA concluded however that CWD was introduced into this Saskatchewan herd in 1995, when 88 elk were introduced from the source herd that was responsible for the overall Saskatchewan outbreak. Based on these investigations, it is unlikely that the export of semen from this herd presented a significant risk of introduction of CWD to New Zealand. (S. Kahn Pers. Comm.2003). Between 1995 and 1999, 4 animals died in the export herd under circumstances that could have been associated with CWD. Although no cases have been diagnosed, surveillance is therefore necessary in New Zealand.

A condition of initially unknown aetiology appeared in farmed elk/wapiti in New Zealand during the late 80s and early 90s. It was characterised by chronic weight loss and was termed "fading elk syndrome". There are no pathological changes in the brain and the laboratory investigations undertaken suggest that the disorder is related to an extreme susceptibility to some strains of abomasal parasites, damage from which results in reduced acid production, raised pH, poor protein digestion and the consequent chronic weight loss. The syndrome has also been recognised in “wapiti” in NA (Waldrup and Mackintosh 1992; C. G. Mackintosh, personal communication).

6 FOOD AND FEED SAFETY AND HUMAN AND ANIMAL RISK

Although CWD is not similar to BSE in terms of epidemiology in that there is no evidence of natural spread of the disease to phylogenetic families other than the cervidae it may theoretically pose a risk for animal and human food safety.

6.1 FOOD SAFETY

There is no evidence that CWD can be transmitted to humans consuming meat or handling infected cervids or their products, however this possibility cannot be ruled out. The World Health Organisation recommends that people not consume animal products from any animal infected with a TSE disease and public health policies in Canada and the US are consistent with this direction.

- In Canada, all adult cervids slaughtered under commercial arrangements in the provinces of Saskatchewan, Manitoba and Alberta are tested for CWD and carcasses are only released upon receipt of a negative result. Offals may be disposed off by incineration or deep burial before test results are known. Once a farmed cervid is diagnosed with CWD, the infected animal and all cervids exposed to positive animals are destroyed and the carcasses disposed of by incineration or deep burial. Antler velvet from test negative animals in the herd is released from official control.
- In NA some health officials advise hunters not to consume meat from animals known to be infected with CWD. In addition, they suggest hunters take simple precautions when field dressing deer or elk taken in areas where the disease is found. Although, in the USA the consumption of meat from CWD affected animals is discouraged, however, there is no ban. So, affected meat probably has been consumed for decades in Colorado and Wyoming (Dr. Williams, Personal communication).
- When an animal is slaughtered in the United States, some animals are tested for CWD depending on individual State regulations. The carcass is stored until result of the test is returned (test takes one week) and when negative then the carcass is released. In case the carcass is positive, further steps will vary from state to state.
- Special slaughterhouses for farmed animals do not always exist: sometimes livestock slaughter plants are used for game animals but they have to handle the different species at different times and clean in between but the rules vary from state to state.
- Fate of road killed deer is not a USDA policy but differs from state to state with considerable variation from location to location.
- Viscera and other carcass remnants of hunted animals remain in situ (offals etc…).

6.2 FEED SAFETY AND ANIMAL HEALTH

Although CWD is not similar to BSE in terms of epidemiology in that there is no evidence of natural spread of the disease to phylogenetic families other than the cervidae it may still pose a risk for animal and human food safety.

- What happens with possible affected carcasses of hunted or slaughtered farmed deer or elk was very much depending on the state, however, the FDA has recently provided new guidance to state public health and agriculture officials throughout the US. FDA does not permit material from CWD positive animals or animals at high risk for CWD to be used as an ingredient in feed for any animal and specifically requiring that renderers do not accept any deer or elk from CWD epizootic areas for any animal feed. This is an extension of the 1997 FDA regulations (feed ban) that put into place the exclusion of ruminant MBM from ruminant feed. This ruling specifically excluded deer and elk from MBM.
- In Canada there are no mandatory controls on rendering carcasses and offal from cervids other than those tested positive for CWD or animals that have been exposed to test positive animals. However, the Canadian Renderers' Association has a voluntary ban on the rendering of cervids. Canada prohibits the feeding of ruminant derived proteins to ruminants.

6.3 CJD IN THE USA AND POSSIBLE RELEVANCE TO CWD

Recently, the Center for Disease Control (CDC) has issued a new statement concerning CWD and possible human infection: "Although it is generally prudent to avoid consuming food derived from any animal with evidence of a TSE, to date, there is no evidence that CWD has been transmitted or can be transmitted to humans under natural conditions". However, the CDC has renewed surveillance efforts in order to rule out a link between CWD and vCJD. While, to date there has been one case of vCJD reported in US (contracted in the UK), the CDC is working with ongoing investigations in Wyoming and Colorado to track cases of CJD or suspected CJD.

CDC reported on the epidemiological investigations carried out on three patients who died of degenerative neurological illness during the period 1993-1999 and who were hunter and/or had a history of consuming venison during wild game feasts. All three patients were aged over 55 and developed neurological symptoms prior to death. Sporadic CJD was confirmed in one case as the cause of death (CDC, 2003).

Belay et al., (2001) Recent reports on of 3 unusually young CJD patients (aged 28, 28 and 30 years) who regularly consumed deer or elk meat, which created concern about the zoonotic transmission of CWD. Investigations, however, by CDC found that these were all cases of sporadic CJD of different types. In the USA the occurrence of CJD in persons 30 years or younger is rare (during 1979-1996, only 12 such CJD cases were reported to the CDC and 8 of these resulted from the use of contaminated growth hormone or dura maters grafts). Against the background of the occurrence and recognition of vCJD in 1996 it is difficult to make an epidemiological distinction between an increased incidence of CJD that might represent an indication of a novel exogenous source of infection and an increased ascertainment of CJD cases in young patients due to better surveillance. Belay et al., (2001), from the National Centre for Infectious Diseases have examined the hypothesis that a causal link could be made between the disease in these 3 patients and CWD. They reviewed medical records and interviewed family members and state wildlife and agriculture officials. Brain tissue samples were examined using histopathologic, immunohistochemical, immunoblotting, or prion gene analysis methods. The investigation assessed the presence or absence of CJD risk factors, associations with deer and elk hunting in CWD- enzootic areas and comparison of the evidence from the 3 patients with that of a zoonotic link between vCJD and bovine spongiform encephalopathy. None of the patients had established CJD risk factors or a history of travel to Europe. Two of the patients hunted game animals and one was a daughter of a hunter. Unlike patients with vCJD, the 3 patients did not express a common phenotype of the disease, which did not suggest a causal link between CWD and CJD (there was also heterogeneity among the three patients on the codon 129 : Met/Met, Val/Val and Met/Val respectively). Molecular phenotyping characteristics for the 2 patients studied gave type 1 on Western Blot according to Parchi et al.(1997), which differs from that of vCJD.

In conclusion, the CDC report on the CJD in the patients aged over 55 years and the investigation of Belay et al. (2001) related to the young CJD patients found no strong evidence for a causal link between CWD and CJD. Both, however, concluded as well that ongoing national surveillance for CJD and other neurological cases will remain important for continuing to assess the risk, if any, of CWD transmission to humans.

Race et al., (2002) described abnormal PrP glycoforms of CWD in comparison to PrP from scrapie and BSE. Analysis of these abnormal PrP glycoform patterns from CWD affected deer and elk, scrapie-affected sheep and cattle and cattle with BSE failed to identify patterns capable of reliably distinguishing these transmissible spongiform encephalopathy diseases. However, PrP-res patterns sometimes differed among individual animals, suggesting the possibility of multiple CWD strains.

7 RISK OF SPREAD TO EUROPE

In answering question two, there was a need for a risk assessment to be carried out and to consider the imports of game meat from cervids and or live animal imports from the USA and Canada, if any.

Data provided by the US and Canada preliminary indicate that there is no export of live cervids into Europe. If licences for export to EU-countries were granted it could be traced back that these were related to single hunter-related trophies (skin, antlers) by private persons. Data provided by Eurostat confirm the data supplied by USA and Canada that there is no import of live cervids into EU countries originating from Canada or US.

Data on game meat provided by Eurostat fall under two different entries related to 'game meat':

- Period 1988-1992 entry is indicated as: 'Fresh, chilled or frozen meat and edible offal of game (excluding of rabbits, hares and swine)'
- Period 1993-2001 entry is indicated as: 'Meat and edible meat offal of game, fresh, chilled, or frozen (excluding rabbits, hares, pigs and quails)'.

A table in Annexe 2 gives the data as provided by Eurostat for the two above-mentioned entries as exported from USA and Canada.

In summary over the period 1988-1992 a total of 0,4 ton and 80,1 ton was exported from Canada and USA respectively to the EU. The exports from Canada were to France, UK and the Netherlands and exports from USA were mainly to UK (79,1 ton) and the rest to France. During the period 1993-2001 a total of 0,7 ton and 120,6 ton were exported from Canada and the USA respectively. The main importer from the USA was UK with 103,8 ton and the remaining importing countries were France Netherlands Germany, Spain and Austria.

There are no data available on exports of cervid embryo's and semen from Canada and the USA and also no import data in the EU. The current, planned and proposed surveillance studies in Europe were discussed earlier. Related to the current activities in Europe further surveillance should be encouraged in order to provide more detailed base line data as the current studies are assessed as insufficient to detect a CWD infection in cervids would it be present.

8 SUMMARY AND CONCLUSIONS

8.1 SUMMARY

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of certain species of native North American deer (mule deer and white–tailed deer, and Rocky Mountain elk. A number of States/Provinces in the USA and Canada, (North America, NA) have reported cases in free-ranging and farmed cervids. There are no reports of CWD in areas outside NA with the exception of a single animal imported into Korea from Canada.

The natural host range of CWD has so far remained confined to cervids. On-going experimental transmission studies have still not been able to show transmission of CWD from deer to cattle. Additionally, genetic studies show a relatively large phylogenetic

difference in PrP sequence between Cervidae, Bovidae and Humans. Those differences suggest an appreciable species barrier for possible transmission of CWD to cattle and humans. However, since the basis of the transmission barrier in relation to the TSE is complex and not solely a function of PrP sequence of donor and recipient it remains theoretically possible that the CWD-agent could infect humans. Infected animals have a widespread tissue distribution of disease-specific PrP and presumably also infectivity in those tissues from an early stage in the incubation period.

Epidemiological data have shown that CWD is readily spread by lateral transmission in cervid populations. In experimental studies, oral exposure to only very small doses of infective material resulted in disease in cervid animals.

Surveillance data do not as yet provide information on accurate figures of the prevalence of the disease in NA and the risk factors are not well understood. Some control measures for farmed deer are in place. However, movement of free-ranging deer provides a major difficulty for control strategies. The origin of the disease is unknown and the lack of any connection with other animal TSEs provides no clues as to the potential for CWD to be pathogenic for man. Available information indicates that there is only negligible trade in live cervids originating in NA to EU but there are indications of imports of small annual tonnage of edible products from game. It is unclear what, if any, trade exists in antler, embryos or semen from cervids between NA and EU countries.

Research and surveillance programs on CWD in farmed or wild Cervidae in Europe did not exist until recently and thus the available data do not allow to draw conclusions about CWD in the Cervidae population in Europe.

8.2 CONCLUSIONS

With regard to the initial question of the mandate, a theoretical risk for prion transmission to humans consuming products of CWD affected-cervids of all ages in countries where CWD exists cannot be excluded. Similarly, transmission risk of prions to domestic animals cannot be excluded. There **is** therefore a scientific basis on which to exclude tissues from animals that carry a CWD risk, from human or animal feed chains.

However, the early and widespread involvement of tissues in CWD infected animals does not allow to define a SRM list, neither to define any lower age cut off as has been

defined for cattle in relation to BSE. Neither is there sufficient knowledge to define exclusions or amendment of any SRM rule on the basis of relative genetic resistance to infection as has been proposed for sheep and goats in the event that evidence indicates the probable natural occurrence of BSE in these species [5].

Although available information indicates imports of live Cervidae from NA to EU and trade in meat products from cervid species as being negligible, it is important to reach certainty that no transfer of risk takes place through trade of live cervids and its derived products.

At present, there are no scientific data that CWD is occurring in Cervidae elsewhere than in those countries from which it has been previously reported. However, systematic TSE surveillance of cervid populations has either been absent or has only just started in European countries. Until results of such surveillance become available no conclusion can be drawn with regard to the occurrence of CWD or similar TSE in the cervid population of Europe.

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10 ANNEXES

Annexe 1

Table: Overview of current information[6] on PrP^{CWD} [7] presence in tissues of mule deer experimentally exposed to the agent of CWD, or with naturally occurring CWD, according to incubation period[8] or age. Note that PrP^{CWD} in elk is not shown since little data up to now are available. (Experimental (oral) study results shown in red)

Tissue	**Mule deer** (15-25m oral experimental incubation)			(Min. 16-17m natural incubation)
	Time post exposure			
	42 days	**2-3m**	**5-6m**	**Clinical (Age)**
Nervous				
Brain (? Including eye)			+	+ (1.5 - 5+yrs)
Spinal cord (T)				+
Spinal cord ©				+ (2.5 - 8+ yrs)
Spinal nerve roots				-
Dorsal root ganglia				-
Myenteric plexus - ??				+
Vago sympathetic trunk				+
Nodose ganglion (Distal G. vagus N.)				+
Brachial plexus				+
Sciatic nerve				+
Trigeminal ganglion				-
Cranial cervical (sympathetic)				-
Coeliac ganglion				-
Anterior mesenteric ganglion				-
Vagosympathetic trunk				+
Sympathetic trunk				+
Lympho-reticular				
Retropharyngeal LN	+			
Parotid LN				+
Mandibular LN				+
Abomasal LN				+
Ruminal LN				+

[6] There are no data on tissue infectivity determined by bioassay for deer or elk other than for brain tissue in clinical cases

[7] From: B. Williams, personal communication, Spraker *et al* 2002a, Spraker *et al* 2002b, Sigurdson *et al* 1999, Sigurdson *et al* 2001, Williams & Miller 2002 and E.S. Williams and M.W. Miller, unpublished.

[8] Only experimental oral exposure study results considered as relevant to natural disease

Mesenteric LN				+
Ileo-caecal LN	+			+
Lymphoid follicles (colon)				+
Inguinal LN				+
Prescapular LN				+
Popliteal LN				+
Tonsil (including pharyngeal tonsil)	+			+ (1.5 - 2.5yrs)[9]
Peyer's patches	+			+
Spleen				+
Alimentary assoc. lymphoid tissue		+	+	
Thymus				-
Bone marrow				-

Table: Overview of current information on PrP^{CWD} presence in tissues of deer and elk with CWD according to age or incubation period

(Experimental (oral) study results shown in red) (Continued)

Tissue	**Mule deer** (15-25m oral experimental incubation Min. 16-17m natural incubation)			
	Time post exposure			
	42 days	**2-3m**	**5-6m**	**Clinical (Age)**
Alimentary				
Tongue				-
Oesophagus				-
Rumen				-
Abomasum (mucosa)				-
Intestines (small & large)				-
Liver				-
Respiratory				
Trachea (epithelium)				-
Bronchi (epithelium)				-
Bronchioles (epithelium)				-
Lung				-
Urogenital				-
Kidney				-
Urinary bladder				-
Ovary				-

[9] Age at which tonsil positive, but brain negative for PrP^{CWD} (Spraker *et al*. 2002b), i.e. possible preclinical cases.

Uterus				-
Endometrium				-
Placentomes				-
Testis				-
Epididymis				-
Endocrine				
Pituitary (pars nervosa)				+
Pituitary (pars anterior)				-
Adrenal (medulla)				+
Pancreas (acinar and islet cells)				+
Thyroid				-
Other				
Myocardium (including Purkinje fibres)				-
Blood vessels walls				-
Skin (epidermis)				-
Sebaceous glands				-
Sweat glands				-
Lacrimal glands				-
Tarsal glands				-
Skeletal muscle				-
Smooth muscle				-

Annexe 2

Table 1 Eurostat data on exports from Canada on ‘game-meat'

FRESH, CHILLED OR FROZEN MEAT AND EDIBLE OFFAL OF GAME
(EXCL. OF RABBITS, HARES AND SWINE) – In TON

Canada EXPORT To :	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
France		0.1												
Netherlands					0.1									
UK		0.2												

MEAT AND EDIBLE MEAT OFFAL OF GAME, FRESH, CHILLED OR FROZEN
(EXCL. RABBITS, HARES, PIGS AND QUAILS) – In TON

Germany												0.4	0.3	

Source: Comext2 k0586711.txt Extracted: 20/11/2002
DataSet: EEC SPECIAL TRADE SINCE 1988

Table 2 Eurostat data on exports from USA on ‘game-meat'

FRESH, CHILLED OR FROZEN MEAT AND EDIBLE OFFAL OF GAME
(EXCL. OF RABBITS, HARES AND SWINE) – In TON

USA EXPORT To :	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
France					1.0									
UK	24.6	21.6	13.6	8.5	10.8									

MEAT AND EDIBLE MEAT OFFAL OF GAME, FRESH, CHILLED OR FROZEN
(EXCL. RABBITS, HARES, PIGS AND QUAILS) – In TON

France									0.5					0.9
Netherlands												1.9	7.2	3.6
Germany								0.6					0.2	
UK						9.3	6.3	5.7	11.5	10.3	12.5	17.7	18.2	12.3
Spain													1.6	
Austria								0.3						

Source: Comext2 k0586728.txt Extracted: 20/11/2002
DataSet: EEC SPECIAL TRADE SINCE 1988