EVALUATION OF THE RISKS TO HUMAN HEALTH FROM THE CONSUMPTION OF FOOD PRODUCTS DERIVED FROM CERVIDS AFFECTED BY CHRONIC WASTING DISEASE

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Evaluation of the Risks to Human Health from the Consumption of Food Products derived from Cervids Affected by Chronic Wasting Disease

The purpose of this report is to evaluate the potential risk to human health from the consumption of food products derived from cervids (deer and elk) affected by chronic wasting disease (CWD). The factors considered in this report include; (i) characteristics of TSE agents; (ii) epidemiology and pathogenesis of CWD and (iii) the potential human health impact from consumption of products derived from CWD-affected cervids.

1 Introduction

Variant Creutzfeldt-Jakob disease (vCJD) emerged as a human transmissible spongiform encephalopathy (TSE) in the United Kingdom in the mid 1990s. Strong experimental and epidemiological evidence indicates that the causative agent of vCJD is the agent responsible for bovine spongiform encephalopathy (BSE) in cattle. It is now accepted that human exposure to the BSE agent was through consumption of contaminated beef and beef products.[1] The ability of the BSE agent to cause disease in humans and the transmission of the agent through the food supply has resulted in worldwide concern regarding the potential human health risks of consumption of products derived from animals suffering from other TSE diseases. In this regard, there has been increased interest in the potential human health impact of CWD, the natural TSE of certain species of cervids.

2 Transmissible Spongiform Encephalopathies

CWD is a member of a group of fatal, neurodegenerative disorders of mammals, called TSEs (also termed prion diseases). Human TSEs include vCJD, sporadic CJD, iatrogenic CJD, Gerstmann-Sträussler-Scheinker (GSS) syndrome, fatal familial insomnia and kuru. The other major TSEs in animals are BSE (cattle), scrapie (sheep and goats), transmissible mink encephalopathy (mink) and feline spongiform encephalopathy (BSE in feline species). CWD is the only TSE to affect free-ranging animals. The TSE group of diseases has been recently reviewed (Lasmezas, 2003).

Although the precise nature of the causative agent(s) of TSEs is still being debated, the hallmark of all prion diseases is the accumulation of a misfolded prion protein (PrP^{D}[2]).

Appendix 1 describes the characteristics of prions and prion strains with particular reference to the prion protein involved in CWD, PrP^{CWD} (refer to footnote #2 for definition).

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[1] In recent months, there has been a second report of human-to-human transmission of vCJD via blood transfusion (Peden *et al*., 2004).

[2] PrP^{D} indicates the disease form of the prion protein. The 'D' superscript is used in this report when the prion protein is discussed without reference to a specific TSE. The superscript can also be used to define specific TSEs. For example, PrP^{CWD} indicates the disease form of the prion protein in CWD. Similarly, PrP^{BSE}, and PrP^{Sc} indicate the disease form of the prion protein in BSE and scrapie respectively. The disease forms of prion protein are generated, through an as yet unknown process, from PrP^{C}, the non-infectious form of the prion protein which is a naturally found in animals. See Appendix 1 for a description of the nature of prion proteins.

3 Chronic Wasting Disease

3.1 Epidemiology of CWD

CWD is a contagious, progressive, neurodegenerative disease affecting free-ranging and captive mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*). CWD is the only TSE to have been detected in free-ranging animals.

The first reported case of CWD was in captive mule deer at a wildlife research facility in northern Colorado in the late 1960s. Although CWD was not reported in free-ranging cervids for another 10 years[3] (Spraker *et al*., 1997), it was estimated that CWD had existed in free-ranging deer populations since the 1950s.

CWD is currently only endemic in North America. For free-ranging cervids, CWD has been reported in parts of Colorado, Wyoming and Nebraska, South Dakota, Illinois, New Mexico, Utah, Wisconsin and Saskatchewan. In farmed cervids, CWD has been reported in Colorado, Nebraska, Minnesota, South Dakota, Montana, Oklahoma, Kansas, Wisconsin, Saskatchewan and Alberta. As of June 2003, brain tissue from more than 111,000 free-range, privately owned or research cervids had been sampled in North America with 629 testing positive for CWD (Bunk, 2004).

Figure 1 is taken from the CWD Alliance website (http://dnr.wi.gov/) and shows the distribution of CWD in free-ranging and captive deer and elk.

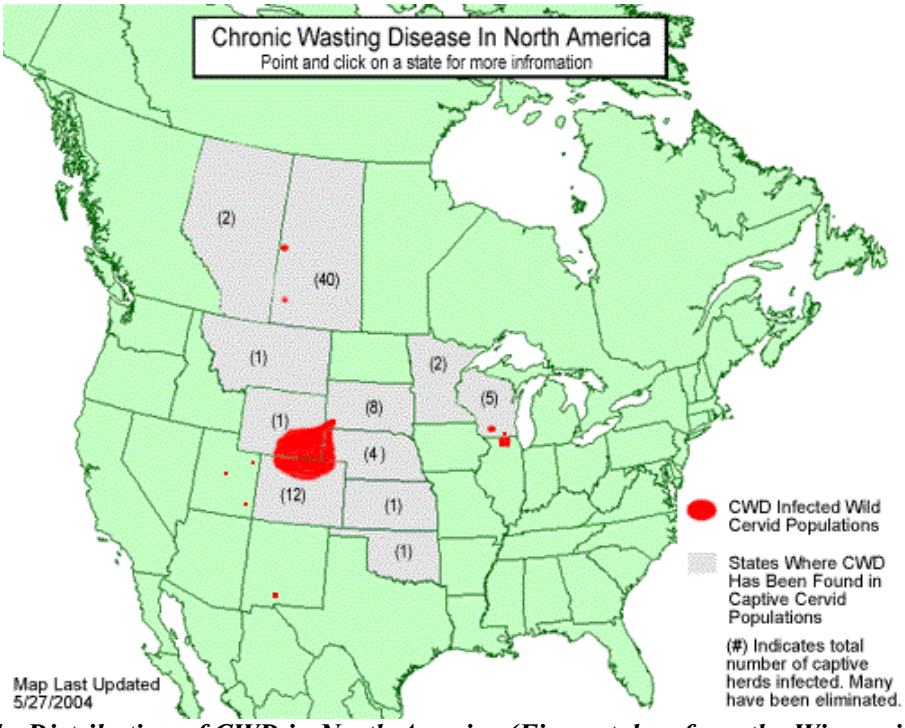

Figure 1. Distribution of CWD in North America (Figure taken from the Wisconsin Department of Natural Resources, http://dnr.wi.gov/)

[3] The first report of CWD in free-ranging cervids was in elk in Colorado in 1981.

3.2 Transmission of CWD

CWD is contagious especially in captive populations, with a reported prevalence of greater than 90% among mule deer in facilities where the disease had been endemic for more than 2 years (Williams and Young, 1992; Williams and Miller, 2002; Miller and Williams, 2003). The contagiousness of CWD is not a common characteristic of TSEs, with the only other contagious TSE being scrapie.[4]

Transmission of CWD occurs horizontally between animals through either direct contact or ingestion of contaminated feed or water (Miller and Williams 2003). Very small doses of infective material are required to induce disease in cervids via the oral route. It has been hypothesised that sub-clinically- and clinically-affected cervids shed the infectious PrP^{CWD} protein through their faeces, saliva and/or urine (Williams *et al*., 2002b; Miller and Williams, 2004). The increase in salivation and urination associated with CWD (as described in Section 3.3) would increase PrP^{CWD} shedding.

Shedding of the infectious agent is presumed to begin early in infection, prior to the development of clinical disease. This is supported by the early detection of PrP^{CWD} in lymphoid tissues of the cervid alimentary tract (tonsils, Peyer's patches and mesenteric lymph nodes) (Sigurdson *et al*., 1999; Spraker *et al*., 2002; O'Rourke *et al*., 2003). It is likely that PrP^{CWD} shedding is progressive throughout the disease incubation period leading to significant environmental contamination (Williams *et al.,* 2002b; Miller and Williams 2004). The environment can also become contaminated from the carcasses of diseased animals. The infectious agent can survive in the environment for at least 2 years and contaminated pasture lands have been implicated as the source of cervid infection in some studies (Miller *et al*., 1998; Williams and Miller 2002).

It is postulated that at least 89% of cases are due to horizontal transmission. Vertical transmission is considered to be a negligible mechanism of transmission (Miller *et al.,* 1998; Miller *et al*., 2000). Transmission via an externally manufactured and supplied food source (such as meat and bone meal) is also considered negligible.

3.3 Pathogenesis of CWD

CWD develops progressively in affected cervids. All animals infected with CWD develop disease. Adult animals 3-5 years of age are most commonly affected, although fawns (<1 year of age) with CWD have also been reported. The oldest animal reported with CWD was over 15 years of age.

The incubation period in free-ranging cervids is not known, however, the mean incubation period in experimentally infected animals averages 15-17 months. Incubation periods vary and may exceed 25 months in deer and 34 months in elk (Williams et al. 2002).

Early clinical signs are subtle and include changes in animal behaviour and temperament. As the disease progresses, clinical signs become more conspicuous and include repetitive behaviours, drowsiness, depression, reduced food consumption, increased drinking, urination and salivation, ataxia and trembling. CWD is always

[4] CWD and scrapie can be transmitted from animal to animal by either direct or indirect contact, although with differing transmission rates. For the other TSEs transmission is either through consumption of infected material (BSE, feline spongiform encephalopathy, kuru and most vCJD cases), treatment-related (iatrogenic CJD), hereditary (GSS, fatal familial insomnia) or sporadic (sporadic CJD).

fatal with death usually occurring within 4 months of the onset of clinical signs, though the clinical disease period prior to death can vary from a few days to more than a year.

There is extensive involvement of multiple organ systems during clinical disease.[5] PrP^{CWD} is found in the brain, spinal cord, eyes, peripheral nerves and lymphoreticular system (Sigurdson *et al*., 1999; Sigurdson *et al*., 2001; Spraker *et al*., 2002). The detection of the CWD agent in tonsils and peripheral nerves early in infection implicates the lymphoreticular system and peripheral nervous system in the dissemination of the infectious agent through the affected animal. Significant pathological changes are confined to the central nervous system and consist of spongiform changes in grey matter, intraneuronal vacuolation, astrocytosis and amyloid plaques. The disease pathogenesis does not differ substantially between captive and free-ranging cervids.

Table 1 lists those tissues in CWD-affected cervids that have been shown by animal bioassay[6] or immunohistochemical studies to contain infectivity (grey shaded lines) (reviewed in Belay et al. 2004).

[5] In this respect CWD is similar to scrapie, where the infectious agent found in multiple organs from scrapie-affected sheep. However, the tissue distribution of the infectious agent in CWD-affected cervids differs from that in BSE-affected cattle where the infectious agent is primarily confined to the central nervous system.

[6] It must be noted that only limited animal bioassays have been undertaken on tissues from CWD-affected cervids and that these bioassays were not undertaken using cervids, which would alleviate any species barrier. However, investigation of infectious bovine tissues from BSE-affected cattle by cattle bioassay has only confirmed the results of the mouse bioassays. Although mice are apparently relatively resistant to CWD, it may be postulated that the use of other non-cervid bioassays, such as ferrets, may be an acceptable and accurate substitute for a cervid bioassay.

Table 1- Detection of PrP^{CWD} in Tissues from CWD-Affected Cervids[△]

Neural Tissues	*Alimentary Tract Tissues*	*Lymphoreticular Tissues*	*Reproductive Tissues*	*Other Tissues*
Brain*	Tongue	Spleen	Testis	Bone marrow
Pituitary	*Salivary glands*	Thymus	Epididymus	Skin
Spinal cord	- mandibular	Tonsil	Ovary	Skeletal Muscle
Retina	- parotid	*Lymph nodes*	Uterus	Myocardium
Ganglia	Oesophagus	- Peyer's patches	Placental tissues	Purkinje Fibres
- optic	Small intestine	- retropharyngeal		Arteries
- coeliac	Colon	- posterior nasal		Veins
- nodose	Pancreas	septum		Thyroid Gland
- dorsal root	Liver	- ileocaecal		Adrenal Gland
Nerves		- mesenteric		Kidney♦
- optic		- sublumbar		Urinary Bladder
- brachial plexus		- popliteal		Trachea
- sciatic		- prescapular		Bronchi
- vagosympathetic trunk		- submandibular		Bronchioles
- myenteric plexus		- parotid		Aleveolar parenchyma
		- ruminal		
		- abomasal		Conjunctiva

[△] Table adapted from (Sigurdson *et al.,* 1999; Sigurdson *et al.,* 2001; Belay *et al*., 2004)
* Shaded cells indicate tissues positive for the CWD agent by immunohistochemical studies. Non-shaded cells indicate tissues negative for the CWD agent by animal bioassay or immunohistochemical studies.

The distribution of PrP^{CWD} outside the brain seems to be less widespread in elk than in deer (Williams and Miller 2002). This may be the reason for clinical disease often being more subtle and prolonged in elk. As with other TSEs, there does not appear to be an antibody or other inflammatory response to CWD (Miller and Williams 2004).

The limited data available regarding CWD suggests that the disease is similar to scrapie in sheep and goats, but different to BSE in cattle. In scrapie the infectious agent is also distributed throughout the lymphoreticular system and peripheral nervous system. Scrapie is also transmitted horizontally from animal-to-animal and may also survive in the environment for prolonged periods. In contrast, the infectious agent has limited tissue distribution in BSE-affected cattle and is transmitted via the feed supply. In spite of the similarities between scrapie and CWD, biochemical studies have shown that the CWD agent is distinct from that which causes scrapie (Raymond *et al*., 2000; Race *et al*., 2002). These studies have also shown that the CWD agent is distinct from that which causes CJD or BSE.

3.4 Genetic Susceptibility of Cervids to CWD

Of the *Cervidae* family only mule deer, white-tailed deer and elk appear to be naturally susceptible to CWD. Other free-ranging ruminants that appear to be naturally resistant to CWD are moose, pronghorn antelope, Rocky Mountain bighorn sheep, mouflon, mountain goats and blackbucks.

The role of genotype in determining susceptibility and resistance of cervids to CWD is unclear with only a few studies having been undertaken. In elk, a methionine at codon 132 is overrepresented in infected animals and is thought to indicate susceptibility to CWD (O'Rourke *et al*., 1999).

In white tailed deer there appears to be a strong association between the allele $Q_{95}G_{96}A_{116}S_{138}$ and CWD and a negative association between the $Q_{95}S_{96}A_{116}$ S_{138} allele and CWD (Johnson *et al*., 2003; O'Rourke *et al*., 2004). It has been estimated that approximately 86-96% of all white-tailed deer in some endemic regions may have the susceptible genotype and therefore be at risk for disease following exposure to the CWD agent.

3.5 Prevention and Control of CWD

There is no treatment or vaccine for CWD currently available. Ante-mortem diagnosis of CWD can be done prior to the emergence of clinical signs, through immunohistochemical examination of tonsil biopsies. Recent surveillance results from the US show that deer with sub-clinical infection can also be detected using immunohistochemistry of lymphoid tissue biopsies.[7] Ante-mortem sub-clinical disease may also be detected using capillary electrophoresis of blood samples (Schmerr *et al*., 1999). Sub-clinical disease is confirmed in ELISA positive animals by the presence of PrP^{CWD} in the obex[8] when examined by immunohistochemistry. Clinical CWD diagnosis is confirmed post-mortem by examination of the brain for spongiform lesions (Williams and Young, 1980; Williams and Young, 1982; Williams and Young, 1993). One of the rapid tests used to diagnose BSE and scrapie[9] has also shown success in diagnosing CWD and has been licensed for use in CWD surveillance in the US and for TSE surveillance in Canada (Wu *et al*., 2004).

Although CWD can be diagnosed in live animals, prevention and control of this disease is problematic due to the ability of the infectious agent to survive in the environment for prolonged periods, and due to the natural migration and movement of affected free-ranging live animals. Control of CWD is through jurisdictional or individual adoption of stringent import requirements, surveillance, quarantine and depopulation, herd certification schemes, and eradication programs for free-ranging cervids. It is not yet clear how effective these prevention and control measures will be.

Other countries have initiated CWD surveillance programs even in the absence of reported cases of CWD. For instance, the European Union is developing a surveillance program for CWD and countries such as the United Kingdom, Germany and New Zealand have already initiated surveillance programs. No infected animals have yet been found.

4 Transmission of the CWD Agent to Other Species

Transmission of TSEs across the species barrier[10] is well documented for some species combinations (Dormont, 2002). Based on the model for species-species

[7] Sampling lymphoid tissue in elk does not have the reliability of sampling lymphoid tissue from deer.
[8] The obex is part of the medulla oblongata in the brain. This region has been used for the diagnosis of TSEs in other animal species.
[9] The Bio-Rad TeSeE® ELISA has been validated and approved for BSE surveillance in the EU and is currently being used in the UK, some EU countries and Japan. The UK is also using this test for scrapie surveillance.
[10] The species barrier determines the relative efficiency with which a disease is passed to an animal of a different species compared to an animal of the same species. Usually it is more difficult and requires a greater amount of infectious agent to infect an animal from a different species compared to an animal from the same species. The species barrier is influenced by the interaction of two variables: the strain

transmission of TSEs detailed in Appendix 2, if a conformation is thermodynamically stable for both cervid PrP^D and the PrP^D for other species then there may be no barrier to transmission and the CWD agent will be able to cause disease in the recipient species. However, if all the conformations of cervid PrP^D that are thermodynamically stable are not stable in any other species, or are less thermodynamically stable in other species, then there will be a significant barrier to transmission and disease is less likely to be induced by the CWD agent.

When the species-species transmission of CWD was examined experimentally, disease was induced in species such as mice, ferrets, mink, raccoons, squirrel monkeys and goats after intracerebral inoculation (Bartz *et al*., 1998; Williams *et al*., 2002a). Cattle are also susceptible to disease when inoculated via the intracerebral route with the one experiment undertaken showing 3 of 13 cattle developing disease after an incubation period of 22-27 months (Hamir *et al*., 2001). Infection was confirmed by immunohistochemistry and Western blot, even though brain lesions were subtle in 2 animals and absent in the third case. The ratio of infected to uninfected cattle indicates susceptibility of cattle to CWD is far less than that observed after scrapie challenge (Cutlip *et al*., 1994).

Although some species are susceptible to CWD when inoculated intracerebrally, there is no evidence for susceptibility by other routes as no prion disease was detected in cattle inoculated orally with the CWD agent and cattle, sheep and goats that have been housed in wildlife facilities in direct or indirect contact with CWD-affected deer and elk have not developed disease (Williams, 2002; Williams *et al.,* 2002a).

4.1 Can the CWD Agent be Transmitted to Humans?

Based on epidemiological and biochemical data, no causal relationship has yet been demonstrated between human prion disease and the consumption of meat products contaminated with the CWD agent (WHO, 2000; Belay *et al*., 2001; Hoey, 2003; Miller and Williams 2004), and there is no other evidence in CWD-endemic areas that CWD poses a human health risk. To date only 2 non-familial CJD cases with a positive history of consumption of venison from CWD- endemic areas have been reported. However, analysis of case details shows that all features of the disease were typical of a sporadic CJD diagnosis. Furthermore, the incidence of CJD and the age distribution of CJD cases in Colorado and Wyoming (endemic CWD areas) are similar to those seen in other parts of the US. Table 2 details the cases of human TSEs that have been investigated for a link to CWD.

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of agent and the donor species effect. Consideration of both of these has lead to the formation of a model of species-species transmission of TSEs. This model is described in detail in Appendix 2.

Table 2. Cases of CJD investigated for possible causal link to consumption of venison from CWD-affected cervids (Belay* et al., *2004).

Age at Death	Year of Death	Consumption of venison from CWD-endemic area	Final diagnosis
25	2001	yes grandfather regularly hunted and shared venison	GSS P120L patient was unusually young
26	2001	no may have eaten venison from non-endemic areas in college	CJD Codon 129[11] = MM PrP-res profile[12] = type 2
28	2002	no may have eaten venison from non-endemic areas at 1-2 years of age	GSS P120L
28	1997	no	CJD Codon 129 = MM PrP-res profile = not done
28	2000	no	CJD Codon 129 = MV PrP-res profile = type 1
30	1999	no	CJD Codon 129 = VV PrP-res profile = type 1
54	2002	no no evidence that the patient hunted in endemic areas	CJD Codon 129 = VV PrP-res profile = type 2
55	1999	no did eat venison from non-endemic areas	CJD Codon 129 = MM PrP-res profile = type 1 most common type of sporadic CJD
61	2000	yes	CJD Codon 129 = MM PrP-res profile = type 1 no atypical neuropathological features
63	2002	no did eat venison from non-endemic areas	CJD Codon 129 = VV PrP-res profile = type 1
64	2002	yes	CJD Codon 129 = MM PrP-res profile = type 1 no atypical neuropathological features
66	2001	no did hunt and eat venison from non-endemic areas	CJD Codon 129 = MM PrP-res profile = type 1

Although the extent that CWD-affected cervids have been consumed is not known, the absence of any associated human disease being detected over the 50 years that

[11] Codon 129 of the human prion protein gene has been linked to susceptibility of humans to TSE diseases. In particular methionine homozygosity (MM) at this codon is linked to susceptibility to vCJD. In comparison, humans with valine homozygosity (VV) or heterozygosity (MV) at this codon appear to be relatively resistant to developing vCJD. Other human TSEs also have genetic mutations that confer susceptibility.

[12] PrP-res is the protease resistant portion of the prion protein. So far, four types of PrP-res have been identified based on their biochemical properties (i.e. glycosylation state, molecular weight).

CWD has been estimated to exist in free-ranging deer populations suggests that there may be a significant transmission barrier between cervids and humans.

In vitro studies support that there is a natural species barrier between cervids and humans that reduces the potential for humans to develop disease after exposure to the CWD agent (Raymond *et al*., 2000). Conversion of human PrP^{C} to the abnormal isoform in the presence of PrP^{CWD} is inefficient compared to the conversion of cervid PrP^{C} by PrP^{CWD} and the conversion of human PrP^{C} by PrP^{CJD} (Raymond *et al.,* 2000). However, cervid PrP^{CWD}-human PrP^{C} conversion was essentially equivalent to ovine PrP^{Sc}-human PrP^{C} but slightly lower than PrP^{BSE}-human PrP^{C} conversion (Raymond *et al.,* 2000). The interpretation of these results is not clear as there is a known link between BSE and human disease but there is no known link between scrapie and human disease. Further work in this area has not yet been published.

Although the above study tends to suggest there is a barrier to transmission of the CWD agent between cervids and humans, data are limited.

To continue to monitor any potential human health impact of CWD, a game hunter registry is operating in Wyoming that will allow for the monitoring and epidemiological follow-up of deaths resulting from a suspected TSE. These investigations will specifically search for any evidence of a rise in the incidence of unusual cases of TSE among hunters that may be related to CWD.

4.2 Measures to Reduce Potential Risk to Humans

Even though there is no evidence to suggest that CWD has been linked to human illness, US authorities have published guidelines to minimise any potential risk of contracting CWD from affected cervids for people who may come into close contact with susceptible animals. Such people include hunters, veterinarians, wildlife biologists, and pathologists. The recommendations are:

1. avoid consuming meat from any deer or elk that looks or acts sick, especially those animals that show obvious signs of emaciation;
2. wear gloves when field dressing or performing necropsy on carcasses, and wash hands and instruments thoroughly with a strong disinfectant when complete;
3. do not consume brain, spinal cord, eyes, spleen, tonsils and lymph nodes from any deer and elk; and
4. thoroughly wash knives and other implements used in processing.

5 Safety of Cervid Food Products

The main food product from cervids is meat. Although there has been recent experimental evidence for detection of the abnormal form of the prion protein in certain muscles of some TSE-affected animals (Bosque *et al*., 1997; Bosque *et al*., 2002), there is no evidence that the muscle tissue from CWD-affected animals contains the infectious prion protein and no evidence that muscle tissue from food-producing animals will transmit infectivity to humans.[13] It is not clear how the research undertaken by Bosque et al applies to naturally-affected animals as the research has all been undertaken in experimentally-infected laboratory animals.

[13] The vehicle for the transmission of the BSE agent to humans is considered to be due to the consumption of beef products which contained infectious tissues (i.e. brain and spinal cord).

Antler velvet is also be used as a dietary supplement and is suggested to have a variety of health benefits. There has been no investigation as to whether cervid antler velvet contains the CWD agent.

If a link between CWD and human illness is established then the safety of cervid food products will depend on identifying the infectious tissues and avoiding cross-contamination of infectious tissues with non-infectious tissues during slaughter and food processing. Minimising cross-contamination is particularly important as the highly stable nature of the infectious agent means routine processing and cooking procedures may not decrease infectivity of contaminated or infectious tissue (refer to Section 3 of Appendix 1 for a discussion of inactivation of TSE agents).

The main organ system involved in cross-contamination in cervids affected by CWD would be the lymphoid system, which has been shown to harbour CWD infectivity and is well distributed throughout the animal. As with sheep it is expected that the removal of lymph nodes from the cervid carcass would be time consuming and not totally effective. A recent report investigating the success of the removal of lymph nodes from ovine carcasses showed that even though more than 99% of lymphoid tissue was removed, two thirds of carcasses still had lymphoid tissue remaining (DNV Consulting, 2001). A similar problem is expected to exist for cervids. Therefore the potential for cross-contamination between infectious lymphoid tissue and apparently 'safe' tissue would be high.

Slaughtering procedures may also be a major source of cross-contamination. Any slaughter procedures using captive bolt stunning would have a risk of cross-contamination of non-infectious tissues, such as the tongue, with infectious tissues, such as the central nervous system. Captive bolt stunning can also cause emboli to dislodge from the brain and travel through the animal, contaminating distal tissues.

Assuming the effective removal of tissues that have been shown to contain the CWD agent from the cervid carcass, it is expected that the risk of exposure to the CWD agent through meat and meat products is negligible. However, any residual CWD infectivity due to cross-contamination will not be eliminated by meat processing procedures.

6 Exposure of the Australian population to the CWD agent

Australia does not currently import live cervids.[14] Uncanned meat products derived from cervids are permitted to be imported into Australia if derived from animals slaughtered, processed, prepared and stored in establishments approved by the Australian Quarantine Inspection Service (AQIS). Currently there are AQIS approved establishments for uncanned red meat products[15] in New Zealand, the United States of America, Canada and Vanuatu. An import permit is required to import product from these countries, with the exception of New Zealand.

[14] Genetic material from cervids are permitted to be imported under certain conditions from New Zealand and Member States of the European Union. Cervine semen has been imported under these conditions.

[15] Uncanned red meat products include fresh meat, biltong, blood, bone, fat, freeze-dried meat, jerky, offal, twiggy sticks and soup products derived from alpacas, antelopes, cattle, camels, cervids, donkeys, horses, goats, sheep, llamas and mules. For cervid species this also includes tail, antlers and velvet.

Over the 2001-2004 period AQIS recorded 385 tonnes of imported cervid meat products into Australia from New Zealand. This figure is based on random surveillance and represents 5% of the total imported product. No meat products specifically identified as cervid, has been imported into Australia from countries apart from New Zealand.

7 Conclusion

This report evaluates the potential risk to humans of the consumption of food products derived from cervids affected by CWD. Evidence for a link between CWD and human disease is lacking, but data is severely limited. It was therefore not possible to determine with confidence whether the CWD agent poses a public health risk.

However, if such a link is established between CWD and human disease then the major issues for food products will be concerning minimising contamination of non-infectious tissues with infectious tissues.

As Australia has only imported cervid food products from New Zealand, which has not reported CWD, the risk to the Australian population from imported cervid food products would be negligible.

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APPENDIX 1: The Nature of Prion Proteins

1. Structure and Function of Prion Proteins

Prion proteins, PrP^C, are normal components of all animals, with the transcription of PrP^C mRNA constitutive in almost all organs (Oesch *et al*., 1991), but particularly in the brain, where the amount of PrP mRNA transcripts is 50 times greater than in other tissues (Oesch *et al*., 1985; Hope and Baybutt, 1991; Oesch *et al.,* 1991). In the brain PrP^C is primarily expressed on the surface of neurons and astrocytes (Oesch *et al.,* 1991). PrP^C expression has also been detected on the surface of keratinocytes (Pammer *et al*., 1998), fibroblasts (Meiner *et al*., 1992) and lymphoid cells (Cashman *et al*., 1990). PrP^C is attached to the cell membrane through a hydrophobic C-terminus and glycosylphosphatidylinositol (GPI)-anchor (Oesch *et al.,* 1991). PrP^C has a long flexible tail (N-terminal region) that can adopt several conformations depending on the physiochemical properties of the extracellular environment (Riek *et al*., 1996; Riek *et al*., 1997).

Although the exact function of PrP^C has not been elucidated, PrP^C has been implicated in regulating neural cell function in relation to circadian rhythms (Tobler *et al*., 1996), synaptic transmission (Kretzschmar *et al*., 2000), neuroprotection (Chiarini *et al*., 2002; Milhavet and Lehmann, 2002) and copper metabolism (Brown, 2003). However, PrP^C is not essential for development or function of the nervous system (Bueler *et al*., 1992). Mice deficient in PrP^C are, however, resistant to TSE diseases illustrating the necessity for this protein in these disorders (Bueler *et al*., 1993; Sailer *et al*., 1994; Brandner *et al*., 1996a; Brandner *et al*., 1996b).

The PrP^C nucleotide sequence in cervids encodes a protein 252 amino acids in length in white-tailed deer and 254 amino acids in length in mule deer and elk. The protein has potential glycosylation sites at amino acids 181 and 197,[16] that allow three different glycosylation states; unglycosylated, monoglycosylated and diglycosylated. However, it has been estimated that, due to the diversity of the glycan side chains, there could be up to 400 different isoforms of the diglycosylated prion protein alone. Experimental analysis by 2D electrophoresis detected over 50 different isoforms of PrP^C in a host, which were defined by glycosylation status and post-translational proteolytic modification (Pan *et al*., 2002).

The consensus pattern of prion proteins is highly conserved across animal species and found in all prion proteins identified. The Internet protein domain and protein family database, PROSITE (PROSITE, 2003), identifies two consensus patterns in the prion protein family.[17] The first is the octapeptide repeat region between amino acids 53-93, which is rich in alanine and glycine amino acids (reviewed in Prusiner, 1998). The number of these repeats differs between and within animal species. The second consensus pattern is centred on the single disulphide bond in PrP^C. The maintenance of this second consensus pattern may define secondary structure required for prion protein activity.

Structurally, PrP^C is composed of three α-helices, comprising approximately 42% of the protein, with minimal β-sheet structure (approximately 3%) (Pan *et al*., 1993). It has been postulated that PrP^C exists as a homodimer through interaction between the α-helices (Knaus *et al*., 2001; Meier *et al*., 2003). In contrast, the disease-specific form of the prion protein, PrP^D, which is implicated as the causative agent in TSEs, comprises approximately 43% β-

[16] Based on the 254 animo acid protein.
[17] PROSITE accession number- PDOC00263

sheets and 30% α-helices (Pan *et al.,* 1993). There are other conformations of PrP^D, which have different proportions of β-sheets.

The conformation of the prion protein appears to be a critical factor in determining the biochemical and physiological properties of PrP^C and PrP^D. For instance, PrP^D has greater biological stability with a biological half-life greater than 25 h, compared to 5 h for PrP^C (Oesch *et al.,* 1991). PrP^D is also insoluble in non-denaturing detergents and is at least partially resistant to protease digestion, whereas PrP^C is soluble and is readily digested with proteases (Dormont, 2002). These characteristics are a function of protein conformation as the higher the proportion of β-sheets in the prion protein, the greater the resistance of the protein to proteases (Aucouturier *et al*., 2000). The resistance of PrP^D to proteases is due to the 27-30 kDa C-terminal fragment, PrP-res. PrP-res is approximately 142 amino acids long and has been shown to induce similar disease as that induced by the entire protein (Bessen and Marsh, 1994).

During progression of the TSE disease, PrP^D acts as a template for conformational conversion of PrP^C to the misfolded PrP^D (Baldwin *et al*., 1994; Cohen *et al*., 1994). This involves conversion of the α-helices to β-sheets and has been postulated to involve the sugar side chains, as glycosylation can interfere with α-helical structure and induce β-sheet formation (DeArmond *et al*., 1997). Conformational change of PrP^C to PrP^D is self-perpetuating and is induced by interactions at the cell surface. The change in conformation is potentially mediated by an unidentified protein, designated Protein X (Telling *et al*., 1995) or by an RNA transcript (Deleault *et al*., 2003).

As PrP^D is relatively insoluble under physiological conditions (i.e. conditions that will not denature the protein) it tends to form aggregates. Aggregation of PrP^D has been shown experimentally to correlate with clinical signs of disease (Xi *et al*., 1992; Demaimay *et al*., 1994; Adjou *et al*., 1995; Adjou *et al*., 1996; Demaimay *et al*., 1997; Adjou *et al*., 2000), and inhibition of PrP^D formation prolongs disease incubation time in TSE-affected mice and hamsters (Xi *et al.,* 1992; Adjou *et al.,* 1995; Adjou *et al.,* 1996).

2. Prion Strains

It is now accepted that it is the structure of PrP^D that differentiates different strains of prion protein (Peretz *et al*., 2002; Somerville, 2002). Different prion strains induce specific clinical and neuropathological patterns in a host species. These strains 'breed true' on repeated passage in the same animal species. This means that similar disease incubation period (Dickinson *et al*., 1968), clinical signs of disease (Pattison and Millson, 1961; Mastrianni *et al*., 1999), lesion profile (Fraser and Dickinson, 1968; Fraser H, 1979) and pattern of PrP^D deposition (Bruce *et al*., 1989; Hecker *et al*., 1992) will occur after serial inoculation of a particular prion strain.

Because prion strains 'breed true' they can be characterised based on disease incubation period, clinical signs of disease, lesion profile and pattern of PrP^D deposition. Prions strains can also be characterised on tertiary structure of PrP^D, glycosylation profile, differential reactivity of PrP^D-specific antibodies, pattern of PrP^D digestion with proteinase K and resistance to chemical or heat inactivation (Collinge, 2001). In addition, strains have been differentiated by Fourier-transform infrared spectroscopy (Caughey *et al*., 1998). Classification of prion strains is incomplete and is difficult, as consideration must be given to the assay and reagents used.

Prion strains will use their conformation as a template for conversion of the PrP^C in the recipient animal to the infectious form. Therefore, even if the PrP amino acid sequences of recipient animals are identical to the donor PrP amino acid sequence, different donor strains may induce PrP^C to misfold into different conformations of PrP^D in the recipient (reviewed in Hill and Collinge, 2002).

There has not been much work investigating whether there are any strains of the CWD agent. However, preliminary biochemical evidence suggests that there may be more than one strain of CWD (Race *et al*., 2002).

3. Physical and Chemical Inactivation of TSE Agents

TSE agents are extremely difficult to physically and chemically inactivate. This is compounded by the fact that different TSE strains or PrP^D from different host species can have different biochemical properties. There are studies investigating inactivation of TSE agents all with slightly different experimental parameters and slightly different experimental outcomes. A summary of the main findings is provided here, with a more comprehensive review in references (Taylor, 2000; Dormont, 2002).

PrP^D has been shown to maintain infectivity after treatment with sterilizing levels of ionising, ultraviolet and microwave radiation. Dry heat is not totally effective at inactivating prions and is highly variable, as different strains of TSE agents have different thermostabilities. Autoclaving, either gravity displacement or porous load cycles, at various temperatures and time settings is also not totally effective at inactivating TSE agents (Taylor *et al*., 1994). In fact, autoclaving at high temperatures has been shown to increase the thermostability of some strains of TSE agents (Taylor and Fernie, 1996).

Treatment with formalin may also increase the thermostability of TSE agents (Taylor, 1989). Treatment with other chemicals, such as acids, alkylating agents, organic solvents or salts has little effect on TSE infectivity. Sodium hypochlorite (25,000 ppm of chlorine) and 4 M guanidine are relatively effective at reducing infectivity of TSE agents, though this may only be partial inactivation. Partial inactivation can also occur after treatment with pronase, trypsin and proteinase K (Prusiner *et al*., 1983) or sodium dodecyl sulfate (SDS). TSE agents have also been shown to maintain infectivity after treatment with nucleases and ethanol and, in the case of scrapie, after burying in soil (Dormont, 2002).

TSE agents can be inactivated by treatment with 1 M sodium hydroxide solution for 1 h at 20°C (Ernst and Race, 1993), or treatment with 2 M sodium hydroxide solution for 30 min at 121°C. A 2% sodium hypochlorite solution for 1 h at 20°C, treatment with saturated phenol or with 10% SDS for at least 30 min can also inactivate TSE agents (Dormont, 2002).

APPENDIX 2: Model for the Species-Species Transmission of TSEs

1. Species-Species Transmission of TSEs

Transmission of TSEs across the species barrier is well documented for some species combinations (Dormont, 2002). The species barrier determines the relative efficiency with which a disease is passed to an animal of a different species compared to an animal of the same species. Usually it is more difficult and requires a greater amount of infectious agent to infect an animal from a different species compared to an animal from the same species. The species barrier is influenced by the interaction of two variables: the strain of agent and the donor species effect. Consideration of both of these has lead to the formation of a model of species-species transmission of TSEs.

2. Donor Species Effect

The donor species effect is determined in part by the nucleotide sequence polymorphisms of the PrP gene in the donor and recipient species. Studies have concluded that the PrP gene and protein that is expressed from this gene is the primary determinant that controls susceptibility of a host species to foreign prions.

The transmission of TSE agents across the species barrier have been mapped to amino acids 108-171 of PrP^C (Scott *et al*., 1993; Priola *et al*., 1994; Kocisko *et al*., 1995; Vorberg *et al*., 2003). The greater the amino acid homology in this region between the infectious PrP^D of the donor species and the PrP^C of the recipient species, the greater likelihood of the recipient species developing disease. The cervid and human forms of PrP share approximately 89% amino acid identity. This is slightly less than that shared by bovine and human PrP (92%). This suggests that transmission of BSE from cattle to humans may be easier than the transmission of CWD from cervids to humans and that there may be an appreciable species barrier for possible transmission of CWD to humans. However, it is more complicated than this, as mentioned in the previous paragraph, the PrP gene is important for determining susceptibility or resistance of a recipient to a TSE. As mentioned in Section 3.4 there are naturally occurring gene polymorphisms in the PrP gene of cervids that appear to confer either susceptibility or resistance to CWD. There are also naturally occurring polymorphisms in the PrP gene of humans that also confer susceptibility and resistance to human TSEs.[18]

Another interesting study has suggested that monogastric species (i.e. humans) are more resistant to TSEs than cervids and other ruminating species (e.g. cattle and sheep) due to the higher gastric acidity in the digestive tracts of monogastric species (Martinsen *et al*., 2002). Further research in this area is not yet available.

3. Model of Species-Species Transmission of TSEs

A model for the molecular basis of prion transmission has recently been reviewed (Hill and Collinge, 2002). The number of highly stable and thermodynamically viable conformations each PrP amino acid sequence is able to adopt is finite. On inoculation of an infectious TSE agent, the conformation of the recipient PrP^C is induced to change to the conformation of the donor PrP^D. If the primary amino acid sequence of the recipient prion protein is not able to sustain the conformation of the donor PrP^D then there will be a substantial barrier to transmission. Alternatively, if the primary amino acid sequence of the recipient PrP allows the protein to fold into the conformation of the donor PrP^D then transmission will be more efficient. Thus, in this model, species-species transmission of TSEs will be determined by the

[18] Humans that are homozygous for methionine at codon 129 are more susceptible to vCJD then heterozygotes or valine homozygotes. Other human TSEs also have genetic mutations that confer susceptibility.

degree of overlap in the conformations allowed by the prion protein of the recipient and donor species. This model is shown diagrammatically in Figure 2.

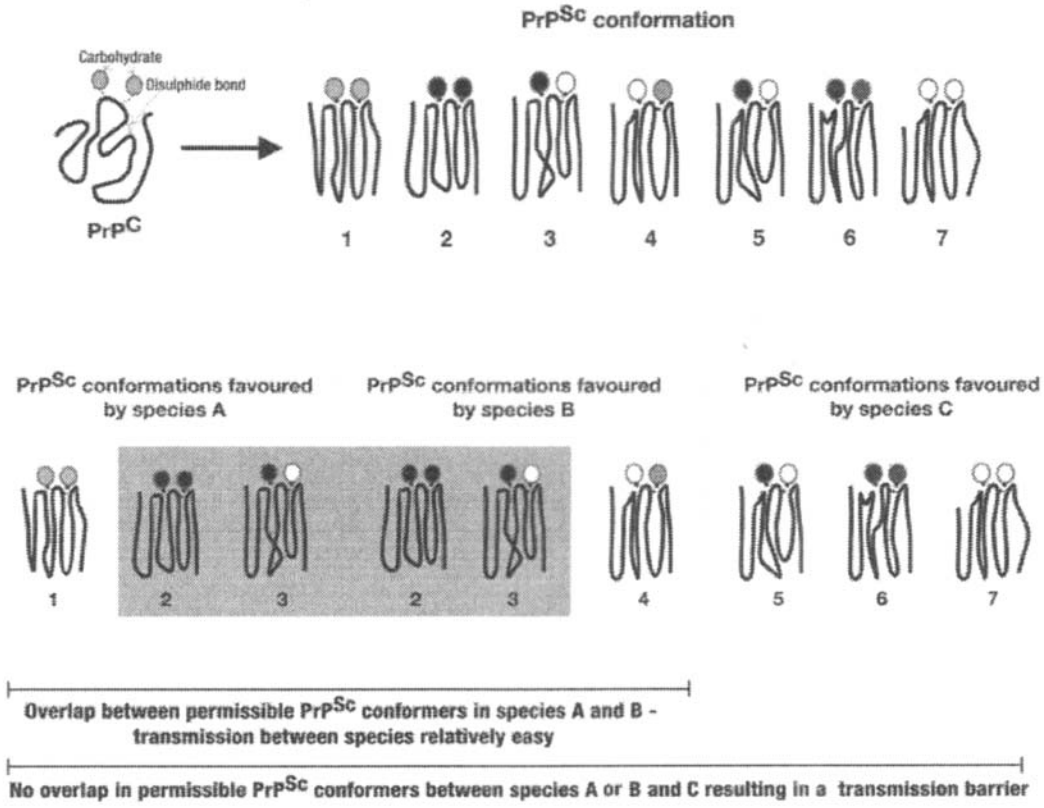

Figure 2. Molecular Basis of Species-Species TSE Transmission
The numbers of PrPD conformations that are highly stable and thermodynamically viable are finite. The particular conformations available for each prion protein will be based on the gene sequence. Transmission will occur between species if the conformations available in the donor species overlap with those available in the recipient species. If the range of PrPD conformations between the two species do not overlap, then a transmission barrier will exist. Figure taken from (Hill and Collinge, 2002).