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Review article

A prion disease of cervids: Chronic wasting disease

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Abstract – Chronic wasting disease (CWD) is a prion disease of deer, elk, and moose, initially recognized in Colorado mule deer. The discovery of CWD beyond the borders of Colorado and Wyoming, in Canada and as far east as New York, has led to its emergence as a prion disease of international importance. Epidemiological studies indicate that CWD is horizontally transmitted among free-ranging animals, potentially indirectly by prion-containing secreta or excreta contaminating the environment. Experimental CWD transmission attempts to other wild and domestic mammals and to transgenic mice expressing the prion protein of cattle, sheep, and humans have shed light on CWD species barriers. Transgenic mice expressing the cervid prion protein have proven useful for assessing the genetic influences of *Prnp* polymorphisms on CWD susceptibility. Accumulating evidence of CWD pathogenesis indicates that the misfolded prion protein or prion infectivity seems to be widely disseminated in many nonneural organs and in blood. This review highlights contemporary research findings in this prion disease of free-ranging wildlife.

CWD / prion / TSE / cervid / amyloid

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1. INTRODUCTION

Prion diseases, or transmissible spongiform encephalopathies (TSE), affect not only domestic and zoo animals, but also free-ranging wildlife. Chronic wasting disease (CWD) is a prion disease of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and Shira's moose (*Alces alces shirasi*) [4, 89, 90], and is only known to occur in North America and in South Korea [16, 39, 40, 93]. That said, international testing for CWD has been minimal with the exception of a CWD surveillance program in Germany [73].

TSE are believed to be caused by the accumulation of a misfolded, β-sheet rich conformer, PrP^{Sc}, of the cellular prion protein,

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PrP^C, which leads to neurodegeneration and ultimately death. In 1978, CWD was first recognized as a new TSE of captive mule deer in Colorado by pathologists Williams and Young [89]. Soon thereafter, a prion disease was reported in elk in Wyoming [90]. CWD was believed to be limited to this central region of the US for two decades. However, within the past 10 years, increased CWD testing has revealed more of the widespread distribution of CWD. Isolated, noncontinguous clusters of prion-infected cervids have been located as far west as Utah and extending east to New York and West Virginia[1]. Local prevalences have reached as high as 30% of free-ranging deer [93]. In Canada, CWD cases have been diagnosed in farmed elk and white-tailed deer, as well as free-ranging deer in Saskatchewan and Alberta [39, 87]. The widespread occurrence of CWD in farmed and free-ranging cervids has led to a surge in CWD research, focused on understanding species susceptibility, transmission and pathogenesis, spatial epidemiology, diagnostic tools, strains, and cervid PrP structure. Transgenic mice expressing cervid PrP have been generated in five laboratories [10, 41, 44, 52, 84], providing useful tools for CWD research. In this review, the latest advances in CWD research are discussed.

2. NATURAL CWD INFECTIONS

CWD is believed to be horizontally transmitted among cervids with high efficiency [54]. Therefore, the capacity for CWD to cross species barriers has been of great interest. Until recently, natural CWD infections were believed to infect only white-tailed deer, mule deer, and Rocky Mountain elk [89, 90, 92]. This ended in 2006, when a report indicated that Shira's moose could be orally-infected with brain homogenate from CWD-infected mule deer, and shortly thereafter a natural case of a CWD-infected moose was discovered [4, 42]. Although moose populations overlap a geographically broad area of endemic CWD, moose are essentially solitary animals with home ranges of 2.2–18.9 km^2 [48] which may help to limit CWD horizontal transmission. Another North American cervid species, the Porcupine caribou (*Rangifer tarandus grantii)*, inhabits Alaska and the Yukon and Northwest Territories of Canada and congregates in dense herds of more than 10 000 animals that can seasonally migrate over 1 000 km. Therefore caribou may be particularly susceptible to rapid disease dissemination since CWD seems to be efficiently transmitted among infected cervids [54]. CWD surveillance of Canadian caribou should be considered for early detection and management, and studies of caribou susceptibility to CWD are warranted.

3. CWD PRION SPREAD AND TARGET ORGANS

Collectively, CWD pathogenesis studies have revealed extensive deposition of PrP^{Sc} in the central nervous system (CNS) and extraneural tissues (Fig. 1). The only other natural prion diseases that even approach this degree of systemic involvement are variant Creutzfeldt-Jakob disease (vCJD) in humans, sheep scrapie, and transmissible mink encephalopathy [22, 23, 30, 61, 62]. In mule deer, PrP^{Sc} is detectable in the retropharyngeal lymph node within only six weeks following an oral exposure [76]. In a further study of the kinetics of prion infection in mule deer, Fox et al. showed that PrP^{Sc} is widely distributed in lymphoid tissues by three months post-oral exposure when it is first detected in brain [17]. By nine months, PrP^{Sc} was detected in the myenteric and submucosal plexi throughout the gastrointestinal tract and in the vagus nerve, and by 16 months, PrP^{Sc} deposits were detectable throughout the brain and spinal cord. The *Prnp* genotype seemed to impact the infection kinetics in that mule deer that were SF heterozygous at codon 225 showed a delay in PrP^{Sc} spread; PrP^{Sc} was not detectable in the brain until 16 months post-inoculation which was 13 months later than the 225SS deer. Perhaps the 225F allele confers a dominant negative effect on the kinetics of this CWD strain, as has been described in sheep, where the 171R allele has been shown to have

[1] http://www.aphis.usda.gov/vs/nahps/cwd/cwd-distribution.html

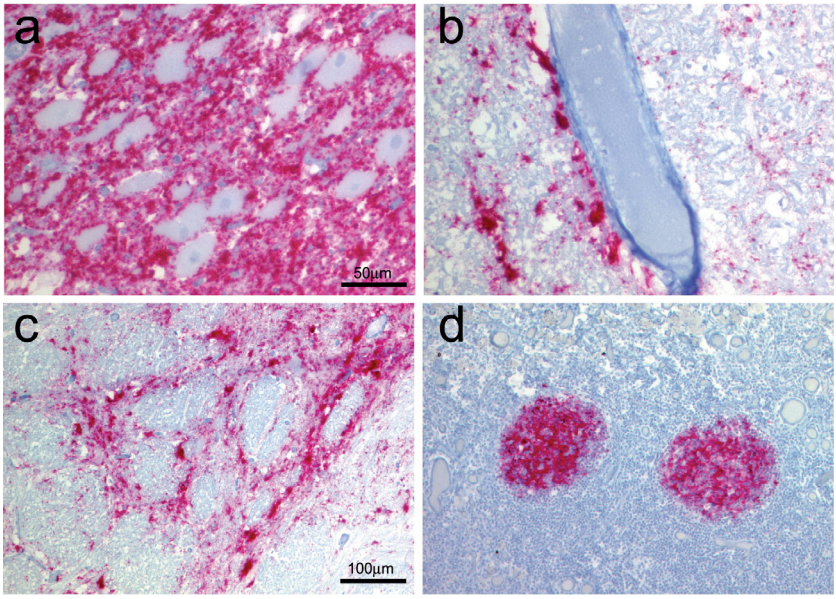

Figure 1. Brain and tonsil sections from a CWD-infected mule deer, immunolabelled for PrP. (a) Brain section at the level of the obex shows the dorsal motor nucleus of the vagus extensively labelled for PrP in the neuropil, however neuronal cell bodies are unstained. (b) Perivascular labelling in the obex is commonly seen in CWD-infected deer. (c) White matter tracts are labelled for PrP. (d) Tonsil contains PrP immunoreactivity within the lymphoid follicles. Uninfected deer show no PrP immunolabelling in brain or tonsils (data not shown). (A colour version of this figure is available at www.edpsciences.org/vetres.)

a dominant negative effect on prion susceptibility [20, 33].

CWD pathogenesis seems to vary between deer and elk: PrP^{Sc} levels have been found to be lower in lymphoid tissues of elk compared to deer [66]. Moreover, in a report of 226 CWD-infected elk, 28 had no PrP^{Sc} in lymphoid tissues despite having PrP^{Sc} in brain [81].

In addition to lymphoid tissues, PrP^{Sc} or infectivity has been detected in other non-CNS tissues, including pancreas [17, 77], adrenal gland [17, 77], and skeletal muscle [2]. Recently PrP^{Sc} was described in cardiac muscle from 7 of 16 (44%) white-tailed deer and from 12 of 17 (71%) elk [35]. This is the first report of PrP^{Sc} in cardiac muscle in any natural TSE.

The cellular and molecular mechanisms of systemic prion spread are under investigation in many laboratories. A recent report showed that blood from CWD-infected deer contained infectivity and could transmit prion disease via a blood transfusion [50]. This finding recapitulates findings of blood infectivity transferred via transfusion from vCJD affected humans [61] and experimental from scrapie sick sheep [32], and indicates that prion transport throughout the body may include the blood as a potential vehicle.

4. THE CERVID *Prnp* GENE

Prnp is highly conserved, and few amino acid residues differ among cervids [1, 34, 58]. However species specific polymorphisms exist, and accumulating evidence suggests that species-specific polymorphisms affect susceptibility to CWD infection with the presently existing strains. In naturally infected mule deer, serine homozygosity at *Prnp* codon 225 seems to increase risk for CWD infection, as the probability that a CWD-infected animal was 225SS was 30 times greater than for S/F or FF when the frequency of genotypes was compared among CWD negative and positive deer (n = 1482 deer) [34]. As noted above,

225SF mule deer had a delay in the kinetics of experimental infection [17].

Elk have a polymorphism at codon 132 (M/L) of *Prnp*, corresponding to polymorphic codon 129 (M/V) in humans [58]. Not only is this codon important for CJD susceptibility and strain typing [86], but it also seems to be influential for elk prion susceptibility. Among free-ranging and captive elk, animals expressing 132MM and 132ML were overrepresented among elk with CWD compared to uninfected controls [58]. In an experimental setting, three of four 132LL elk orally infected with CWD developed clinical disease by 59–64 months post-inoculation whereas 132MM or 132ML elk ($n = 2$ each) developed terminal clinical prion disease by 23 or 40 months post-inoculation, respectively, confirmed by immunohistochemistry and Western blot for PrP^{Sc} [27, 60]. Therefore the 132 LL and 132ML heterozygous elk had a 2–3 fold delay in the infection kinetics, indicating that the 132 polymorphism may influence prion conversion in elk.

White-tailed deer also have a polymorphism at *Prnp* codon 96 (S/G) [36]. Transgenic mice expressing cervid PrP of either allelic variant, denoted here as tg(CerPrP96G) and tg(CerPrP96S), were exposed to deer CWD [52]. Whereas the tg(CerPrP96G) mice were highly susceptible to infection and developed disease at 160 days post-infection, the tg(CerPrP96S) mice were completely resistant, with no clinical disease or PrP^{Sc} deposits even at 600 dpi. It is possible, however, that the tg(CerPrP96S) mice may be converted by deer CWD derived from an animal that was SS homozygous at codon 96. In naturally infected white-tailed deer, deer that expressed 96SS PrP had a lower risk for CWD infection, but were not resistant, since at least three of 7350 CWD infected deer were positive for PrP^{Sc} in brain [36, 59]. Other studies suggest that the 96S allele delays CWD disease progression [95]. Perhaps these two allelic genotypes select for different CWD strains.

5. PRP^C STRUCTURE

The PrP^C structure has been solved by nuclear magnetic resonance (NMR) analysis from recombinant prion protein from a library of vertebrate species [13, 21, 31, 45–47, 63]. The global architecture of the various mammalian PrP structures are nearly identical. Intriguingly, elk PrP possesses an extremely well-defined loop connecting the second alpha helix and beta sheet (amino acids 166–175), whereas the homologous region is flexibly disordered in human and bovine PrP^C [21]. In the laboratory of Kurt Wüthrich, this loop region has been studied in detail, and the loop is thought to provide structural insights into species barriers for prion disease [12]. Further structural studies in two mutant mouse PrP variants derived from the elk PrP primary structure, mPrP[N174T] and mPrP[S170N, N174T], have confirmed that the defined loop in the elk is due to two amino acid exchanges, as the mPrP[S170N, N174T] has the conformationally identical rigid loop of the elk [21]. It is of great interest to determine whether the loop region of the elk influences TSE susceptibility and CWD transmission to other species.

6. CWD STRAINS AMONG DEER AND ELK

Prion strains, such as those seen in sheep scrapie, show distinct incubation periods in differentially susceptible inbred mice and lesions target discrete brain regions [11, 18, 19]. CWD in deer and elk has been considered a single disease entity, and Western blot glycoform patterns of PrP^{Sc} are similar among deer and elk [67]. However, some new data indicate otherwise, suggesting that conformational variants, or strains, may exist. In a study by Raymond et al., Syrian golden hamsters were infected with mule deer or elk CWD, but with an incomplete attack rate; only 2 of 7 and 0 of 7 hamsters developed terminal disease, respectively. Indeed, second and third passage of the mule deer derived strain resulted in a short incubation period of only 85–89 days, whereas the elk-derived strain led to an incubation period of 408–544 days. Surprisingly, when mule deer CWD was first passaged in hamster PrP expressing transgenic mice and then into hamsters, a slowly replicating strain with a distinct clinical disease and PrP^{Sc} deposition patterns in brain ensued. Therefore, two different strains could be passaged from

a single mule deer CWD isolate, a rapid and a slowly replicating strain with differing disease phenotypes [70]. Alternatively, these strains could have been generated upon interspecies transmission [6].

We have also observed two strains arising from a single CWD-infected mule deer upon passage in transgenic mice overexpressing murine PrP. Here, mice developed different PrP^{Sc} aggregate morphologies in brain, either dense, congophilic plaques or fine, diffuse aggregates which could be selectively passaged [78]. LaFauci et al. have reported that elk PrP expressing transgenic mice developed phenotypically divergent diseases when inoculated with either mule deer or elk CWD, which was suggestive of different strains among cervids [44]. In some of these studies, it is not clear whether mule deer and elk possess heterogeneous PrP aggregates (strain mixture), or whether the strains may have developed in the new host. However, Safar et al. have reported differing conformational characteristics for PrP^{Sc} from CWD-infected white-tailed deer and elk directly, using a conformation dependent immunoassay (CDI) [71], which supports the existence of CWD strains. The possible existence of CWD strains is perhaps not entirely surprising, considering that there are genetic *Prnp* differences among deer and elk that could influence PrP^{Sc} conformation [34, 36, 58].

7. INTERSPECIES CWD TRANSMISSION

Wild predators and scavengers are presumably feeding on CWD-infected carcasses. Skeletal muscle has been shown to harbor CWD prion infectivity [2], underscoring that other species will almost certainly be exposed to CWD through feeding. However, CWD has not been successfully transmitted by oral inoculation to species outside of the cervid family, suggestive of a strong species barrier for heterologous PrP conversion. Ferrets (family Mustelidae) can be infected with deer CWD after intracerebral (ic) but not oral exposure [5, 80]. Raccoons resisted even ic infection for up to two years thus far [24]. Mountain lion (*Puma concolor*) susceptibility to experimental feeding of CWD prions is currently under investigation (M. Miller and L. Wolfe, personal communication).

Could wild rodents colonizing CWD- or scrapie-infected pastures serve as an environmental reservoir of prion infectivity? Interestingly, bank voles (*Clethrionomys glareolus*), are readily infected with CWD and sheep scrapie by intracerebral inoculation ([64]; U. Agrimi, unpublished data) and are considered as a potential reservoir for sheep scrapie [64]. Many vole species occur in North America [65,83] and further research may determine whether voles enhance CWD or scrapie spread through environmental contamination.

Given that environmental contamination with CWD prions likely occurs [55], domestic ruminants may be exposed to CWD through common grazing areas. However, sheep and cattle appear to be poorly susceptible to mule deer CWD: ic inoculation with mule deer CWD succeeded to infect only 2 of 8 sheep [28]. Likewise, cattle have note proven to be infected after six years of co-grazing with CWD-infected mule deer, or six years following a direct oral exposure (six years post-inoculation) (M. Miller, personal communication). Even direct ic inoculation led to CWD infection in only 5 of 13 cattle (38%) after 2–5 years [26]. In contrast, cattle are highly susceptible to white-tailed deer CWD with 12 of 14 animals developing neurologic disease and PrP^{Sc} by only 22 months post-ic inoculation (±0.5 months) [29]. Further studies are planned to determine whether cattle are susceptible to white-tailed deer prions after an oral exposure (J. Richt, personal communication). The differential susceptibility of cattle to CWD from mule deer versus white-tailed deer suggests that CWD strains exist, and that CWD may differentially cross species barriers depending on the strain. Nevertheless, to date, natural CWD infections have been detected only in cervids.

Is the converse true, are cervids susceptible to prions from other species? Only one study has been performed on cervid susceptibility to sheep scrapie by the ic route, and showed that 3 of 6 elk developed neurologic signs, spongiform encephalopathy and PrP^{Sc} in brain [25]. Further experiments to address this question

may be interesting since sheep scrapie is considered a possible source for CWD in North America [89, 91].

8. HUMAN SUSCEPTIBILITY TO CWD

Millions of North Americans hunt deer and elk (US Department of the Interior, Census Bureau), and there is no doubt that people have been exposed to CWD through venison consumption, particularly in light of recent data showing CWD prions in muscle [2]. Human susceptibility to CWD or to other newly emerging animal TSE [9,14] is still unclear, although we can be somewhat reassured in that there have been no large scale outbreaks of human TSE cases in Colorado and Wyoming, where CWD has existed for decades [51]. Up until approximately 10 years ago, autopsies were not performed on suspect human TSE cases in many states due to biosafety concerns, therefore the diagnosis of potential new TSE strains has been hampered. This indicates that clinical TSE diagnoses in humans were not confirmed, nor was any strain typing done to look for the appearance of potentially subtle or unusual pathological or biochemical phenotypes of a new TSE strain. Fortunately, the autopsy rate for suspect cases is improving. At the National Prion Disease Pathology Surveillance Center at Case Western Reserve University (Cleveland, Ohio), Creutzfeldt-Jakob disease (CJD) suspect cases are studied and classified by CJD subtype. Thus far, twenty-seven CJD patients who regularly consumed venison were reported to the Surveillance Center, however there have been no unusual or novel prion subtypes that might indicate the appearance of a new prion strain [7, 41].

Other indirect studies of human susceptibility to CWD also suggest that the risk is low. In biochemical conversion studies, Raymond et al. [68] showed that the efficiency of CWD to convert recombinant human PrP into amyloid fibrils was low, but similar to that of both BSE and scrapie fibrils to do the same. These results suggest that there is a molecular incompatibility in the conversion of human PrP^{C} by CWD, sheep scrapie, or BSE, and that cross species infections in humans may be rare events.

To determine whether common PrP^{Sc} strain features may link CWD and CJD, histopathology and the PrP^{Sc} biochemical characteristics from deer and elk were compared with that of humans with sporadic CJD (sCJD) cases that are methionine homozygous at codon 129 of the *Prnp* gene by Xie et al. [96]. However, strain features including histologic profile, target organs, and glycoform patterns will not necessarily remain the same upon crossing species barriers [5, 6, 8, 57]. The PrP^{Sc} form is cleaved by proteinase-K (PK) at different sites depending on the conformation of the protein and may aid determination of whether the PrP^{Sc} conformation is similar. By Western blot (SDS-PAGE) of elk CWD, the unglycosylated PK-resistant PrP^{Sc} migrated to 21 kDa, similar to sCJD (MM1 subtype) and the PK cleavage site was the same, occurring at residues 78 and 82 as assessed by N-terminal sequencing. Conformational stability was evaluated by measuring the PrP^{Sc} stability under partially denaturing conditions and also showed no significant difference between elk CWD and sCJD MM1 PrP^{Sc}. However, elk CWD and human sCJD MM1 strains exhibited distinct glycoform patterns by two dimensional gel electrophoresis, suggesting that the strains differed. Future studies may incorporate use of luminescent conjugated polymers, which were recently shown to distinguish naturally- and experimentally-derived prion strains [79].

To study elk-human prion species barriers, Kong et al. inoculated elk CWD into transgenic mice expressing either human PrP or elk PrP. Whereas the elk PrP expressing mice developed disease after only 118–142 days post-inoculation, human PrP expressing mice (129M) did not develop any features of TSE after more than 657 or more than 756 days [41]. In accordance with these results, Tamgüney et al. also reported that human PrP overexpressing mice were not susceptible to nine CWD isolates from mule deer, white-tailed deer, and elk [84]. However, mice have a limited lifespan and further passages may be necessary to detect low levels of prion pinfectivity that may be present subclinically.

Although indirect evidence is accumulating that there may be a robust species barrier

for CWD transmission to humans, one report indicates nonhuman primate susceptibility to CWD. Intracerebral inoculation of squirrel monkeys (*Saimiri sciureus*) demonstrated a positive CWD transmission [49]. Among nonhuman primates, however, the *Prnp* sequence of the new world monkeys are the most distant from humans [72], and therefore may not indicate that human prion protein conversion would be induced by CWD PrP^{Sc}.

9. CWD DIAGNOSTICS

Clinical signs of CWD in deer and elk are unspecific and subtle in early disease and commonly include weight loss and behavioral changes such as isolation from the herd and depression. Other signs may include hypersalivation, polydipsia/polyuria, ataxia, and occasionally increased regurgitation and/or esophageal distension. Therefore sensitive, specific, and rapid ante-mortem CWD assays are critical for accurate diagnosis. Removal of infected animals from a herd, particularly in US National Parks where culling of only known infected individuals is often the preferred method of management (M. Wild, personal communication). To survey and manage CWD in Rocky Mountain National Park and adjacent Estes Park in Colorado, mule deer were anesthetized, radiocollared, and tonsil biopsies collected and tested (M. Wild, personal communication; [94]). Any prion-infected animals were then located by radiotelemetry and euthanized. Therefore, intensive CWD management can be costly and labor intensive.

Rectal biopsies have been evaluated as an alternative to tonsil biopsies for large scale surveillance of free-ranging or captive deer. PrP^{Sc} was readily detected by IHC in rectal lymphoid follicles from experimentally infected deer, where 19 of 19 deer were positive by one year after oral inoculation. In naturally-infected mule deer, 45 of 50 subclinical and terminal CWD-infected deer, as determined by immunohistochemistry on tonsil or retropharyngeal lymph nodes, were also positive by rectal biopsy [95]. Rectal biopsies to diagnose CWD in elk may also be suitable, as one study showed a strong correlation between tonsil, rectal mucosa, and brain in CWD positive elk, although one of seven elk with early CWD was tonsil positive, but rectal mucosa negative [82].

PrP^{Sc} detection in CWD-infected deer blood has been a challenge to develop as an antemortem diagnostic tool. PrP^{Sc} was recently reported as detectable in deer blood using an antibody ELISA with signal amplification of antibody conjugated DNA catalyzed by T7 RNA polymerase [15].

Protein misfolding cyclic amplification (PMCA) can be used to amplify PrP^{Sc} from CWD-infected deer, and should be valuable for PrP^{Sc} detection from tissues and body fluids containing low levels of infectivity, for improved understanding of CWD pathogenesis, and also to detect animals in early stages of infection [43]. Atarashi et al. [3] developed a highly sensitive PMCA amplification technique whereby recombinant, natively folded PrP monomers is used to amplify PrP^{Sc}; as little as 50 ag of PrP^{Sc} was detectable. Therefore methods for sensitive detection of PrP^{Sc} are substantially improving.

10. TOOLS FOR CWD RESEARCH: CELL LINES AND CERVID PrP EXPRESSING TRANSGENIC MICE

Until recently, few techniques were available to detect CWD prion infectivity, or to test compounds to disrupt prion conversion. A CWD-susceptible cell line derived from cervid brain fibroblasts has been used to screen inhibitors of CWD infection, for example, pentosan polysulfate [69]. This CWD specific assay may identify compounds that inhibit CWD propagation.

Browning et al. developed the first transgenic mouse expressing cervid PrP [10], which was subsequently utilized as a bioassay to detect CWD prions in muscle [2]. Several transgenic mice expressing cervid PrP have since been produced using different constructs and sequences [10, 41, 44, 52, 84] (detailed in Tab. I). The cervid PrP-expressing transgenic mice provide an important tool for investigations of CWD and other TSE, particularly for cross-species transmission studies. In a study by Trifilo et al. cervid PrP expressing mice

Table I. Transgenic mice developed for CWD research.

Prnp gene[1]	Lines[2]	Promoter	Expression[3]	Cervid CWD: primary passage[4]	Average incubation period (days)[5]	Reference
Mule deer	Tg(CerPrP)1536$^{+/-}$	Hamster prion	5X WT	MD, elk	230–260	[10]
Mule deer	Tg(CerPrP)1534$^{+/-}$	Hamster prion	3X WT	MD	270	[10]
Deer (96G)	Line 33$^{+/-}$	Mouse prion	> deer	WTD, MD, elk	200–400	[52]
Deer (96G)	Line 15$^{+/-}$	Mouse prion	1X deer	WTD, MD, elk	160–450	[52]
Deer (96G)	Line 39$^{+/-}$	Mouse prion	1X deer	WTD, MD, elk	200–400	[52]
Deer (96S)	Line 60$^{+/-}$	Mouse prion	> deer	WTD, MD, elk	resistant (> 600 days)	[52]
Deer (96S)	Line 80$^{+/-}$	Mouse prion	1X deer	WTD, MD, elk	resistant (> 600 days)	[52]
Elk	TgElk$^{+/+}$	Hamster prion	2.5 WT	MD, elk	95 (MD); 130 (elk)	[44]
Elk	Tg(ElkPrP)12577$^{+/-}$	Hamster prion	2X WT	WTD, MD, elk	180 (WTD); 200 (MD); 185 (elk)	[84]
Elk	Tg(ElkPrP) 12580$^{+/-}$	Hamster prion	2X WT	Elk	205	[84]
Elk	Tg(ElkPrP)3934$^{+/+}$	Hamster prion	3X WT	Elk	145	[84]
Elk	Tg(ElkPrP) 12584$^{+/-}$	Hamster prion	3X WT	Elk	150	[84]
Deer	Tg(DePrP)10945$^{+/-}$	Hamster prion	1X WT	WTD, MD, elk	400 (WTD); 340 (MD) 290 (MD); 330 (elk)	[84]
Deer	Tg(DePrP)10969$^{+/-}$	Hamster prion	1X WT	MD, elk	325 (MD); 305 (elk)	[84]
Elk	Tg12$^{+/-}$	Mouse prion	2X WT	Elk	120–140	[41]

[1] All cervid constructs are 96G except for the two 96 S transgenic mice produced by Meade-White et al. [52]. All deer constructs are 138S. All elk constructs are 132M.
[2] All mice are on a *Prnp*$^{0/0}$ background. Transgene homozygosity is indicated by $^{+/+}$, and hemizygosity as $^{+/-}$.
[3] WT indicates wild-type murine PrP levels.
[4] WTD = White-tailed deer; MD = mule deer.
[5] Incubation period defined as time from inoculation to terminal disease.

were orally susceptible to CWD, enabling kinetic studies of CWD pathogenesis [85]. All together, these experiments demonstrate the utility of these mice as a tool for studying CWD transmission, species barriers, and understanding the pathogenesis of CWD through tissue and body fluid bioassay.

11. DISEASE CONTROL CHALLENGES POSED BY CWD

Evidence is building that indicates efficient horizontal transmission occurs in CWD, indeed a complicating aspect in disease control [91]. Potential transmission mechanisms

range from spread via direct contact among animals to environmental exposure through grazing in areas contaminated by prion-infected secretions, excretions (saliva, urine, feces), tissues (placenta), or decomposed carcasses. Recently, in a breakthrough finding, saliva from CWD-infected deer was shown to transmit prion disease [50]. An additional experiment by Miller et al. showed that CWD-infected carcasses allowed to decay naturally in confined pastures can lead to CWD infections in captive deer, demonstrating the potential for environmental contamination to spread infection [55]. Modelling studies have provided further support that environmental contamination is likely playing a significant role in transmitting CWD [53, 56]. Additionally, infectious prions have been demonstrated to bind soil particles and remain infectious to animals by both intracerebral and oral exposure routes [37, 38]. Prion infectivity has been recovered from soil more than two years after experimental exposure to prions, suggesting the soil may serve as a reservoir for CWD prions [75]. Taken together, these results indicate that there may even be multiple sources for CWD exposure, perhaps through direct contact and environmental routes.

Significant challenges to CWD eradication exist in free-ranging cervids. Infected deer and elk range over a broad geographic region, and even previously surmised geographic barriers such as the Continental Divide have proven passable by infected animals. Ridding the environment of CWD-contaminated soil or even CWD-infected carcasses is not possible. Moreover, the available ante-mortem diagnostic tests for surveillance are laborious and impractical for large numbers of free-ranging animals [74, 88, 95]. Therefore for a wildlife manager, this disease is costly to survey and difficult to control.

12. CONCLUSION

CWD in cervids is efficiently transmitted, likely more than any other TSE in animals or humans. Therefore, it is unlikely that this TSE can be eradicated. Perhaps through an improved understanding of transmission routes, biological factors influencing pathogenesis, and the molecular basis of CWD prion conversion, a targeted strategy for interrupting disease spread may be developed.

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