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Chronic wasting disease

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Abstract

Until recently, chronic wasting disease of cervids, the only wildlife prion disease, was believed to be geographically concentrated to Colorado and Wyoming within the United States. However, increased surveillance has unveiled several additional pockets of CWD-infected deer and elk in 12 additional states and 2 Canadian provinces. Deer and elk with CWD have extensive aggregates of PrP^{Sc} not only in the central nervous system, but also in peripheral lymphoid tissues, skeletal muscle, and other organs, perhaps influencing prion shedding. Indeed, CWD is transmitted efficiently among animals by horizontal routes, although the mechanism of spread is unknown. Genetic polymorphisms in the *Prnp* gene may affect CWD susceptibility, particularly at codon 225 (S/F) in deer and codon 132 (M/L) in elk. Since CWD infects free-ranging animals and is efficiently spread, disease management will be a challenge.

A chronicle of CWD

A prion disease of free-ranging wildlife, chronic wasting disease (CWD) affects mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) [1], and moose (*Alces alces shirasi*) [2], all members of the family Cervidae. CWD was first noted in 1967 within a research facility in Fort Collins, Colorado where captive mule deer used for nutrition research were reported with a body wasting syndrome [2]. After more than a decade of uncertainty about the etiology of CWD, pathologists Elizabeth Williams and Stewart Young recognized the brain lesions as those of a transmissible spongiform encephalopathy (TSE) in 1978, and CWD was subsequently demonstrated as a prion disease not only by the classic neuronal perikaryonic vacuoles [3], but also by the accumulation of aggregated prion protein [4] (Fig. 1) as well as prion infectivity in the brain [5]. In the late 70s and early 80s, CWD was detected in two zoological collections, in Wyoming and in Canada [6]. Beginning in 1981, cases of CWD were discovered in wild deer and elk on the eastern slope of the Rocky Mountains and extending out on the plains following river valleys within Colorado and Wyoming [7,8]. By 1996, CWD was first detected in Canada's farmed elk, and soon thereafter in the US elk industry, although it may have occurred in this industry far earlier. More recently, CWD-infected ranched elk have been discovered in several other US states and in South Korea [9] [10] raising international awareness and concern regarding CWD. The origin of CWD remains an enigma.

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Epidemiology and disease management

Based on published and unpublished estimates, there may be well over 30 million cervids in North America [11]. Prior to 2000, it was known that CWD had spread in part through transport of captive deer and elk and movements of free-ranging animals, but its distribution was believed to be limited to a 40,000km^2 region of northern Colorado and southern Wyoming [1,8], with a small number of cases in Canada [1,6]. CWD surveillance has recently been undertaken in other states and provinces, and the results have been astonishing. CWD-infected cervids have been reported in 12 additional states, extending east to New York and West Virginia, as well as in 2 Canadian provinces (http://www.aphis.usda.gov/vs/nahps/cwd/cwd-distribution.html;http://www.nwhc.usgs.gov/disease_information/chronic_wasting_disease/north_america_CWD_map.jsp) (Fig. 2). The distribution pattern is not in contiguous zones consistent with natural movement of free-ranging animals, but instead concentrated in focal hotspots of varied size separated by large distances (Fig. 2). Wisconsin has dense white-tailed deer populations (15–20 deer/km2) with a prevalence of up to 13% of the male deer in some regions [12]. The origins of these recent outbreaks remain under investigation, but in some cases spillover from infected game farms seems a plausible explanation. The appearance of CWD in wild cervids presents significant challenges to disease control or eradication due to (i) the extensive geographic range of North American deer and elk, (ii) the logistical difficulty in applying ante-mortem diagnostic tests such as tonsil biopsy [13], and (iii) the inability to rid the environment of potential prion contaminated excreta.

CWD surveillance in Europe has been more limited, however some countries such as Germany have conducted an active surveillance program. In Germany, a total of 7300 captive and free-ranging cervids were tested for CWD with no sign of infection [14]. Reindeer or caribou *(Rangifer tarandus)*, from North America or Northern Europe respectively, have a highly homologous prion sequence compared with mule deer, thus are likely susceptible to CWD. Other European cervids such as moose and red deer *(Cervus elaphus)* are also expected to be CWD-susceptible.

CWD infects free-ranging animals, creating an enormously complex situation for controlling disease spread, particularly in light of our poor understanding of specific transmission routes and susceptibility of non-cervid species. In addition, prion-infected deer and elk will be consumed by scavengers and other carnivores, including mountain lions, foxes, raccoons, coyotes, as well as eagles and vultures. Domestic ruminants and other herbivores are likely exposed through CWD contaminated grazing areas, and conversely, wild ruminants are likely exposed to sheep scrapie. Species known to be susceptible to CWD by an extreme and unnatural exposure route, intracerebral inoculation, include ferrets [15], raccoons [16], other ruminants (discussed below), and squirrel monkeys [17]. Studies are ongoing to determine whether mountain lions (*Puma concolor*) are susceptible (M. Miller, pers. communic. and [18]).

Transmission among cervids

Of all the mammalian prion diseases, CWD is likely the most efficiently transmitted. In dense free-ranging deer populations, CWD prevalence can reach as high as 30%, however in captive herds, prevalence can climb to nearly 100% [2]. How is CWD transmitted with such efficiency? This question is arguably one of the biggest conundrums in the CWD field, and hypotheses range from spread via direct contact to exposure through grazing in areas contaminated by prion-infected secretions, excretions (saliva, urine, feces), tissues (placenta), or decomposed carcasses. Indeed, Miller et al. have shown that CWD-infected carcasses allowed to decay naturally in confined pastures can lead to CWD infections in captive deer [19]. Perhaps multiple exposure pathways can lead to an infection, nevertheless, horizontal spread of CWD is clearly

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occurring [20] and vertical transmission cannot be excluded. Conceivably the abundant PrP^{Sc} in tonsils and Peyer's patches [4] contributes to PrP^{Sc} shedding in saliva or feces. Nonetheless, environmental prion contamination underscores the difficulties of CWD eradication, a goal which is unlikely to be met for free-ranging cervid populations with the available management techniques. Indeed, CWD eradication is not a stated management goal for all western states.

Inflammation may increase the risk of prion shedding in cervids. In prion-infected mice, follicular inflammation in the kidney directs prions to accumulate within lymphoid follicles [21] and intriguingly, leads to prion excretion into the urine of infected mice [22]. Even in natural sheep scrapie cases, follicular mastitis results in prion accumulation in the mammary gland [23]. It remains to be seen whether scrapie prions are then shed into the milk to infect nursing lambs. Because deer and elk also have a widespread prion assemblage within lymphoid tissues, it seems plausible that follicular inflammation may also lead to CWD prion build-up in nonlymphoid organs, potentially shifting shedding routes. It is unknown whether other types of inflammation, such as the granulomatous inflammation in the intestine seen in Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) (affects ruminants, including deer and elk) or parasitic inflammation could lead to or perhaps increase prion excretion by fecal routes.

Recent studies of prion disease in hamsters indicate the potential for prion shedding via saliva [24]. In hamsters intracerebrally (ic) exposed to scrapie, prions are transported centrifugally from brain to the tongue, and PrP^{Sc} deposits in muscle, nerve, taste buds, and epithelium, serving as a large potential reservoir for continual PrP^{Sc} shedding into saliva. In addition, PrP^{Sc} has been detected in the tongues of 7 of 10 sheep naturally infected with scrapie using both western blotting and immunohistochemistry techniques [25]. However, the tongue of CWD infected deer and elk has not yet been investigated for the presence of PrP^{Sc} or prion infectivity. Nevertheless, CWD-infected tonsils contain abundant PrP^{Sc} (Fig. 1) [26] and may also serve as a source for prion shedding into saliva. In light of the commonly shared salt licks and water sources in captivity, as well as licking behaviors of deer and elk, PrP^{Sc} transmission via saliva should be considered as suspect.

Transmission to livestock

The capacity for CWD transmission to other species is clearly an area of great concern since potentially CWD-infected free-ranging animals are co-habitating with domestic ruminants. However, data on the risk for other wildlife species or domestic ruminants contracting the disease is steadily accumulating. Cattle have been challenged with CWD by 3 routes: (i) intracerebral, (ii) oral, and (iii) via contact exposure to CWD-infected mule deer (co-habitation) [25, M. Miller, pers. communic]. After 6 years, only cattle challenged by ic inoculation have developed disease. Five of thirteen animals (38%) developed prion infection after an incubation period of 2–5 years [27]. Secondary passage of the cattle CWD led to a decrease in incubation period to ~16 months with 100% attack rate (n=6) [28]. Perhaps surprisingly, cattle did not develop a spongiform encephalopathy, although PrP^{Sc} was clearly detected in brain by immunohistochemistry and Western blot. By comparison, ic sheep scrapie infection in cattle resulted in 100% of cattle developing neurologic disease with PrP^{Sc} deposits in the brain (9/9) [29]. A targeted surveillance of 262 older cattle from a CWD endemic area in Colorado did not reveal any indication of a TSE [30].

Sheep are also susceptible to CWD after intracerebral inoculation [2], but have not yet been challenged by oral routes. One goat developed TSE six years after CWD inoculation and showed signs of intense pruritis and weight loss [6]. Elk have been challenged with sheep scrapie, and developed a spongiform encephalopathy with PrP^{Sc} in the brain detected by immunohistochemistry and western blot. Intriguingly, the histologic lesions and PrP^{Sc} deposits

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in the brain were indistinguishable from CWD in cervids [31]. As far as we know, deer have not been directly challenged with sheep scrapie, however this experiment may be interesting and at least addresses whether sheep scrapie could be an origin for deer CWD.

CWD and human susceptibility

Several million deer and elk hunters consume venison in the US and Canada and there is no doubt that people have been exposed to CWD. Human susceptibility to CWD is still unclear, although we can be somewhat reassured in that there have been no large scale outbreaks with hundreds of human TSE cases in Colorado and Wyoming, where CWD has existed for decades. That said, diagnosis of potential new TSE strains has been hampered in that, up until recently, autopsies were not performed on suspect human TSE cases in many states due to biosafety concerns. 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), 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 indicating the appearance of a new prion strain. [11,32]

Other indirect studies of human susceptibility to CWD, although limited in number, suggest that the risk is low. In biochemical conversion studies, Caughey et al. 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 [33]. Recently Xie et al. have compared histopathology and PrP^{Sc} biochemical characteristics from deer and elk with that of humans with sporadic CJD cases that are methionine homozygous at codon 129 [34]. The PrP^{Sc} form is cleaved by proteinase-K at different sites depending on the conformation of the protein so can be used to aid determination of whether the PrP^{Sc} conformation is similar. For CWD, the unglycosylated PK-resistant PrP^{Sc} migrated at 21 kDa, similar to sCJD (MM1 subtype), the PK cleavage site was the same, occurring at residues 78 and 82 as assessed by N-terminal sequencing, and the conformational stability also showed no significant difference between elk CWD and sCJD MM1 cases. However, there were distinct glycoform patterns exhibited by two dimensional gel electrophoresis, suggesting that the elk CWD and human sCJD MM1 strains differ, although strain features, including histologic profile, target organs, and glycoform patterns, will not necessarily remain the same upon crossing species barriers [15, 35,36].

Kong et al. studied the question of human susceptibility to CWD by inoculating transgenic mice expressing human PrP or elk PrP with elk CWD. 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 >657 or >756 days [11].

Cervid prion genetics

The deer and elk primary protein structures are highly conserved as seen in other mammals. There are four particularly intriguing features of the deer and elk prion gene. First, a polymorphism at codon 225 (S/F) may influence CWD susceptibility. When comparing the frequency of genotypes among CWD negative and positive free-ranging mule deer (n=1482), the odds that a CWD-infected animal was 225SS was 30 times greater when compared to 225SF [37].

Second, elk have a polymorphism at codon 132 (M/L) of *Prnp*, corresponding to polymorphic codon 129 (M/V) in humans. Elk expressing 132MM and 132ML *Prnp* were reported to be

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overrepresented among elk with CWD when compared to uninfected controls [38], and 132LL elk experimentally infected with CWD have resisted infection for at least 4 years, 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} [39].

Third, white-tailed deer also have *Prnp* polymorphisms which may affect CWD susceptibility. When the allelic frequencies from CWD-positive and CWD-negative free-ranging, Wisconsin white-tailed deer were compared, a G96S and a Q95H polymorphism were linked to a reduced susceptibility to CWD [40].

A fourth interesting feature of deer and elk prion genetics from an evolutionary perspective is the pseudogene which has been described in mule deer [41] and white-tailed deer [40,42]. The pseudogene is suspected to be a processed retrotransposon, since it lacks introns and is flanked by direct repeats. In WTD, the pseudogene encodes five or six octapeptide repeats [42]. At residue 138, the *Prnp* functional and pseudogene diverge, encoding a serine or asparagine, respectively [42]. Neither Old World Rocky Mountain elk nor New World moose possess the pseudogene, perhaps indicating that the *Prnp* pseudogene arose after evolutionary radiation of *Odocoileus* in the New World [42].

Cervid PrP structure

The structure of the elk prion protein has been solved by NMR analysis of recombinant elk PrP [43] (Fig. 3). When the elk PrP structure is compared to human or bovine PrP structures, the global architecture is nearly identical. However, intriguingly, the elk PrP possesses an extremely well-defined loop connecting the 2^{nd} alpha helix and beta sheet (amino acids 166–175), whereas the homologous region is flexibly disordered in human and bovine PrP^{C}. This loop region has been studied in detail in the laboratory of Kurt Wüthrich, as there is an outstandingly high incidence of nonconservative species variation and the loop is thought to provide structural insights into species barriers for prion disease [44]. Further structural studies in 2 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 2 amino acid exchanges, as the mPrP[S170N, N174T] has the conformationally identical rigid loop of the elk. Whether this loop region confers any TSE susceptibility or pathologic consequences remains to be established.

Structural differences clearly influence species susceptibility, a feature well known for sheep scrapie where susceptibility is heavily influenced by genotype at codons 136 (V/A), 154 (H/R), 171 (Q/R) [45]. One proposed etiology of prion disease is based on a natural propensity for PrP to assume beta-sheet-rich conformations, in combination with a failure to prevent the accumulation of the beta-rich isoform, thereby ultimately leading to aggregated PrP [46,47]. Interestingly, the amyloidogenic site of the yeast prion protein, Sup35, has the same amino acids as in the loop region of elk PrP, only the sequence order is different, but possibly suggests an inherent propensity for a beta-rich conformation with these particular amino acids [48]. Based on this unusual rigid structural feature of the elk PrP, it is tempting to speculate that the isolated geographic foci of CWD outbreaks across the U.S. and Canada may be due to increased risk for a sporadic disease that can then spread horizontally. At least such possibilities should remain open to discussion. Another possibility for the isolated outbreaks would be the undetected spread of CWD through commerce of captive cervids.

Clinical disease and lesions in natural cases

Deer and elk with CWD show subtle signs of disease, characterized by weight loss, isolation from the herd, hypersalivation, polydipsia/polyuria, frequent regurgitation ± esophageal

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distension, and rarely ataxia [49] (Fig. 4). The clinical signs are nonspecific and difficult to detect in early stages except by those working daily with the animals. In one report, a captive herd of 133 white-tailed deer was discovered with 50% CWD positive in brain or lymphoid tissue, though no clinical signs were noted by the landowner or hunters [42]. The clinical course varies from several weeks to up to many months [50].

Necropsy findings include subacute to chronic bronchopneumonia (likely due to aspiration), froth or watery rumen contents (often containing sand), abomasal or omasal ulcers, serous atrophy of bone marrow and pericardial fat, enlarged adrenal glands, and muscle atrophy [7].

Histopathologic lesions in the brain are similar to those described for BSE in cattle and scrapie in sheep and goats: perikaryonic neuronal vacuoles, microcavitation of gray matter, astrogliosis, neuronal degeneration and loss, and PrP^{Sc} positively labeled prion deposits and plaques. Target areas for severe spongiform change included olfactory bulb and stria, septal nuclei, thalamus, supraoptic and paraventricular nuclei, tegmental nuclei, and within the medulla oblongata, neurons of the reticular formation, as well as nuclei from the hypoglossal, vagal, medial and lateral cuneatus, and spinal tract of the trigeminal nerve [4,7,50]. Neuropathology varies slightly between deer and elk: elk have more severe lesions in the thalamus and in some white matter areas [50]. Congo red bifringent, PAS-positive amyloid plaques have been seen in deer brain but not elk [50].

Pathogenesis

Transmission of CWD into deer and elk demonstrated CWD as a prion disease, with spongiform change and PrP amyloid fibrils in the brain of inoculated animals [2,6]. Oral CWD infection of mule deer fawns indicates that PrP^{Sc} appears at early timepoints in the retropharyngeal lymph node, tonsil, and Peyer's patches by 3 months post feeding [51]. At terminal stages of disease, PrP^{Sc} accumulates in part in the intestinal plexi and vagus nerve, although the timing of prion infection of ganglia and nerves is not known [52].

CWD is one of several prion diseases in which PrP^{Sc} is typically disseminated throughout the lymphoid system prior to the CNS. Thus, tonsil biopsy can be used to diagnose CWD ante-mortem, although a negative tonsil biopsy does not rule out CWD infection as not all follicles are necessarily positive [51,53] and not all CWD cases have a lymphoid phase of infection. This scenario is seen particularly in elk, where 28/226 CWD positive elk in one study did not have detectable PrP^{Sc} in the lymphoid system [54]. PrP^{Sc} in lymphoid tissue co-localizes with follicular dendritic cell (FDC) and tingible body macrophage markers [55], as seen with natural sheep scrapie and experimental mouse scrapie [56,57]. In mouse scrapie models, C1q is essential for peripheral PrP^{Sc} replication, and C1q deficiency leads to a marked delay in incubation period, suggesting that the classical complement cascade is an important early step in prion pathogenesis [58,59]. The mechanism by which C1q interacts with PrP^{Sc} and the connection with accumulation on FDCs is unresolved, but may also have relevance for CWD pathogenesis.

Early stages of oral uptake of prions across the intestinal mucosa of sheep have been recently examined in greater detail by Jeffrey et al [60]. Loops of intestine were isolated and inoculated with prions. PrP^{Sc} was detected in the dilated villous lacteals and submucosal lymphatics by 3 hours post-inoculation, within dendritic-like cells in the draining lymph node by 24 hours, and in the Peyer's patches only by 30 days post-inoculation. Uptake was not affected by the *Prnp* genotype of the sheep. It is not known precisely when and how prion infectivity enters nerves, or whether PrP amplication in the Peyer's patches is a prerequisite for nerve entry, although Mabbott et al. have shown in mice that prion infection of Peyer's patches is irrelevant to CNS infection as early as 14 days post-oral challenge [61].

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In studies by Spraker et al., PrP^{Sc} accumulation in the brain has been characterized in great detail in MD, WTD, and elk, both captive and free-ranging [4,7,54]. Experimental CWD time course studies are underway but have not yet been published to characterize the pathogenesis, therefore it remains unclear when and how PrP^{Sc} reaches the brain and spinal cord. However, natural CWD cases in MD have been classified by PrP^{Sc} and spongiform encephalopathy distribution throughout the brain [62]. PrP^{Sc} consistently accumulated in the brainstem, particularly the dorsal motor nucleus of the vagus. Other organs that accumulate PrP^{Sc} in mule deer include adrenal glands, pancreatic islets, and pituitary [52].

New tools for CWD investigation

A big leap forward for CWD research occurred with the development of cervid PrP expressing transgenic mice (CerTgPrP), recently been described by Browning et al. to be highly susceptible to CWD infection [5]. Mice develop plaques in the brain resembling CWD in cervids. Thus far, prion infectivity in various organs of cervids has not yet been examined in detail by bioassay, however, CWD infectivity has been recently shown in skeletal muscle of naturally CWD-infected deer [63]. A second cervid PrP transgenic mouse has been described by Kong et al. [11].

A CWD-susceptible cell line has been developed derived from cervid brain fibroblasts, and has been used to screen inhibitors of CWD infection, e.g., pentosan polysulfate [64]. This is the first CWD specific assay introduced for screening compounds that inhibit CWD propagation. Perhaps these cells will also be useful to assess CWD infectivity.

CWD strains?

In sheep scrapie, many strains of TSEs exist, including an ‘atypical scrapie’ known as Nor98 [65]. For CWD, the existence of multiple strains is unclear. Transgenic mice overexpressing murine PrP and inoculated with CWD develop a mixture of pathological and biochemical phenotypes which may suggest the existence of multiple strains, although the phenotypic variability could also be due to strain adaptation (CJS and AA, unpublished data). Safar et al. reported differing conformational characteristics for CWD from WTD and elk using a conformation dependent assay (CDI), also suggestive of strain differences [66].

In light of the discussion herein, one aspect of CWD research is crystal clear: there are many unsolved mysteries in this disease of wild animals. The sooner we understand basic disease factors such as CWD origins, mechanisms of spread, and species susceptibility, the more specifically we can target prevention and management programs.

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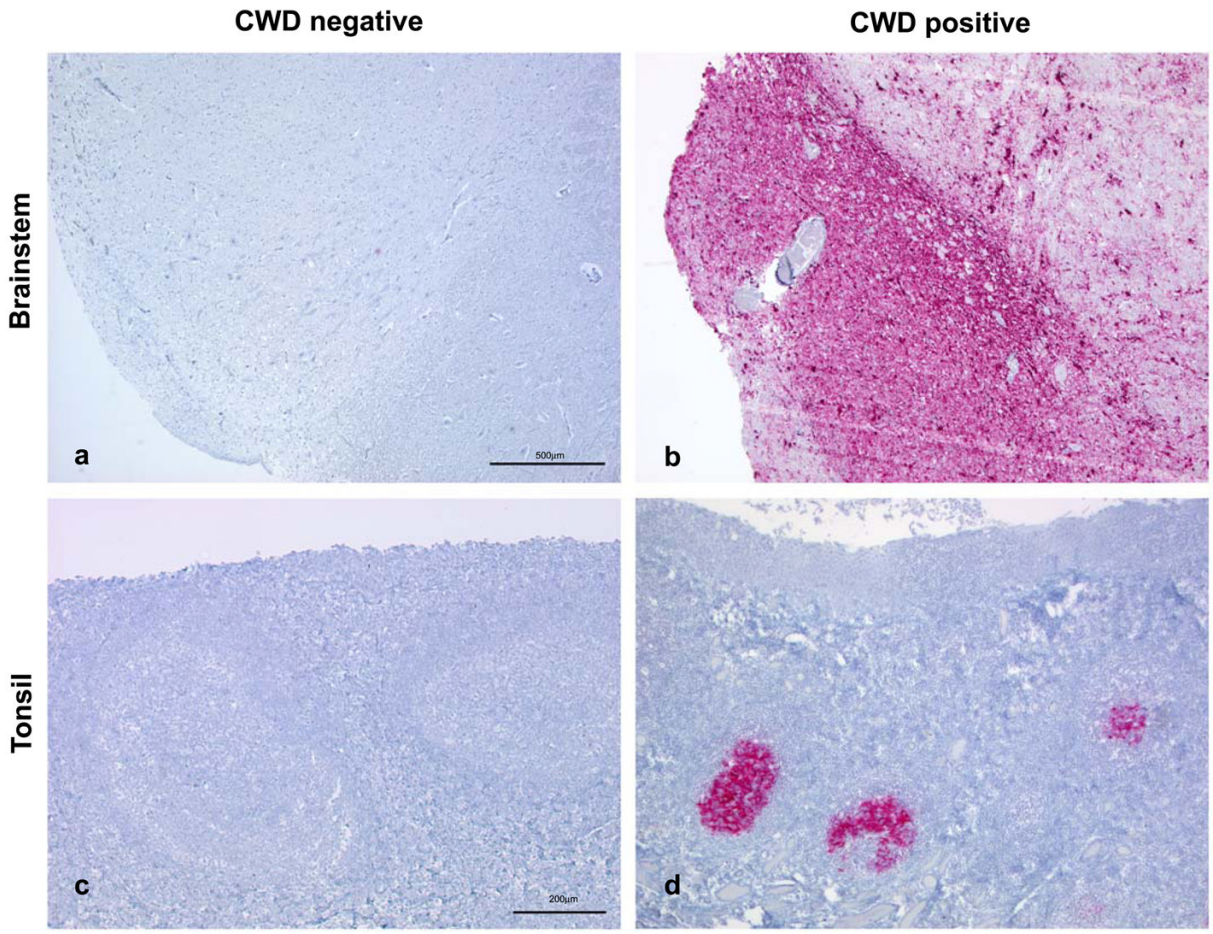

Figure 1.
Immunohistochemistry of mule deer brain and lymphoid tissue using anti-PrP antibody F99/97.6.1 [67]. PrP^{Sc} deposits in the brainstem and tonsil from a CWD-infected mule deer (panels b, d), but not in an uninfected deer (panels a, c).

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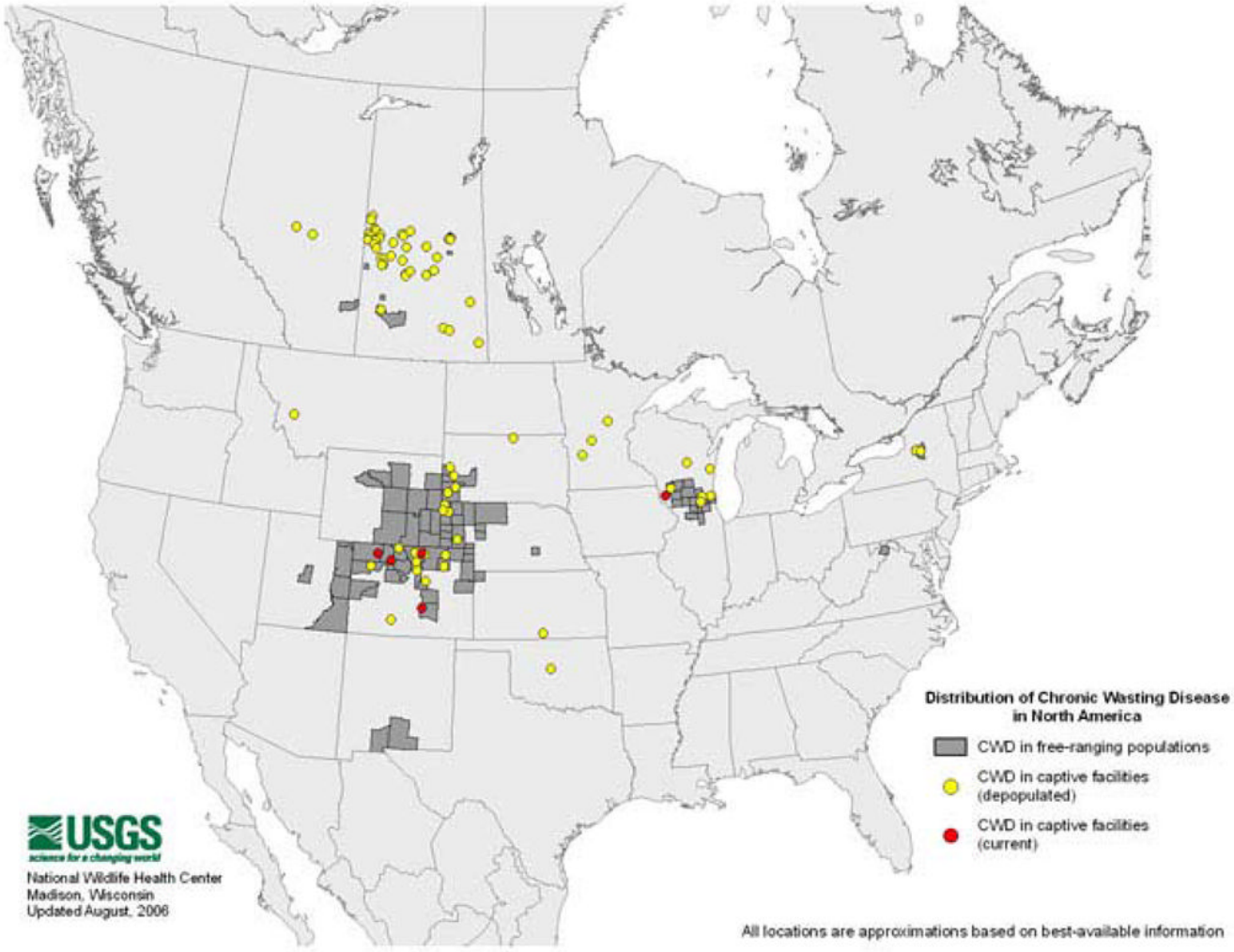

Figure 2.
Map depicting the CWD cases detected in North America. (Map kindly provided by the National Wildlife Health Center).

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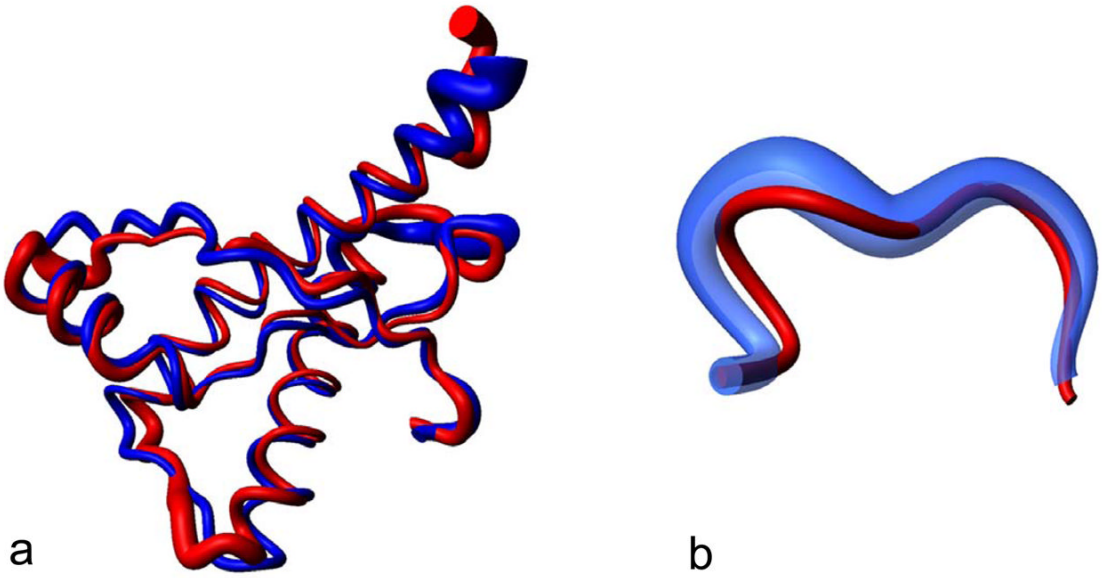

Figure 3.
Superposition of the mean NMR structures of the polypeptide segment 125–229 (C) and 165–172 (D) in ePrP(121–230) (red) and mPrP(121–230) (blue). A spline function was drawn through the C positions. The radius of the cylindrical rods representing the polypeptide chains is proportional to the mean backbone displacement per residue (XX), as evaluated after superposition for best fit of the atoms N, C, and C′ in the 20 energy-minimized conformers used to represent the NMR structures [43,68]. Figure provided by Simone Hornemann. Data published in reference [43,68].

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Figure 4.
Mule deer with clinical signs of CWD including hypersalivation, a lowered head, emaciation, and a dry, rough hair coat (panel a). A clinically normal control mule deer is shown for comparison (panel b).

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Table 1

Inter-ruminant prion transmission experiments.

Host	Inoculum	Exposure route	# Infected/# Exposed	Time to terminal disease	Spongiform encephalopathy	PrP^{Sc} detected by IHC and/or WB	Reference
Cattle	Mule deer CWD+ brain	Intracerebral (ic)	5/13	2–5 years	Equivocal	Yes	27
Cattle	Cattle CWD from ic infection above	Intracerebral	6/6	16.5 months	No	Yes	29
Elk	Sheep scrapie	Intracerebral	3/6*	3–4 years	Yes	Yes	31

* intercurrent death in 3 animals during first 2 years post-inoculation