Chronic Wasting Disease: Violating the Laws of Biology

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Introduction

Chronic Wasting Disease (CWD) is a member of the Transmissible Spongiform Encephalopathies family of diseases and presents unique challenges to researchers and conservationists due to its atypical nature. Affecting members of the cervid family, CWD is characterized and induced by a misfolded endogenous prion protein, PrP^{CWD}. As CWD transmission has been occurring at an exponential rate, it has become a topic of interest for many to understand the exact nature and transmissibility of this disease.

Transmissible Spongiform Encephalopathies

Transmissible spongiform encephalopathies (TSEs), commonly referred to as prion diseases, are a group of lethal and highly transmittable diseases characterized by the malformation of endogenous prion proteins (PrP) [1-3]. Located primarily in the brain, properly folded prion proteins (PrP^{C}) have been found to reside on cellular membranes, yet their functionality is currently unknown [4]. Researchers have hypothesized the role of PrP^{C} lies in some form of protection from oxidative damage and maintaining synaptic homeostasis due to its copper binding nature [1]. The protein misfolding responsible for inducing TSEs is characterized by the transformation from a primarily alpha helix secondary structure to majority beta sheet, causing additional tertiary structure changes without any change in the amino acid sequence. When the structural change is induced, the now malformed PrP is released from the cellular membrane into the cytosol. Once in the cytosol, the damaged PrP collects in the lysosomes until

the cell lyses releasing damaged PrP, subsequently inducing changes in the neighboring cells and coalescing into amyloid plaques like those seen in Alzheimer's disease. This structural change and subsequent plaque formations render the damaged PrP immune to proteolysis, a process further outlined in the glossary [1, 3-7].

The observed confirmation shift is thought to be caused by consuming a damaged PrP that likely induces the PrP^{C} to change secondary structure via electrostatic and steric interactions between the two protein molecules [3-7]. This cascade is observed at an exponential rate, with the initial low rate of induction being responsible for the latency period observed in all forms of TSEs [8]. This protein only theory of replication directly violates the widely accepted theory that all genetic information is encoded in a form of nucleic acid [9]. The importance of this distinct trait of TSEs will be discussed further in relation to Chronic Wasting Disease (CWD) in later sections.

Due to genomic data researchers have been able to identify that approximately 15% of prion diseases seen are due to a vertically transmitted mendelian disorder or spontaneous mutation associated with one of the more than thirty recognized coding mutations in the prion gene PRNP, while the remaining 85% are considered sporadic, resulting from random mutations or consumption of infected materials [1, 9]. As illustrated in Table 1 there are currently eleven main forms of TSEs, five human TSEs and six animal TSEs [8]. The remainder of this paper will discuss the animal TSE Chronic Wasting Disease and its effects on various ecosystems.

Chronic Wasting Disease

Chronic Wasting Disease, a TSE affecting members of the cervid species such as deer, elk, and reindeer, caused by a mutated PrP (PrP^{CWD}) poses a growing threat to ecosystems and

agricultural institutions around the world. CWD is characterized in cervids by weight loss, polyuria, torpor, low urine specific gravity, polydipsia, hypotonia, wasting, general motor impairment, and ataxia [3,4,6] First recorded in Colorado in 1967, CWD near immediately became endemic [3,5]. Being the only form of animal TSE that is present in both captive and wild populations of cervids, CWD presents a unique containment problem for ecologists and researchers. While all non-mendelian variants of TSEs display a high level of transmission, CWD is considered unparalleled in its efficacy of transmission [10,11].

As seen in Figure 1, CWD has spread at an exponential rate from 2000 to current day [12]. As of April 2019, there have been cases of CWD reported in either free range or captive populations of cervids localized across 23 states in America, with additional reports of infected individuals in South Korea and Norway [3,11,12]. The cases that have appeared in South Korea have been hypothesized to be resultant of transferring infected animals not yet exhibiting symptoms to game parks throughout South Korea. Additionally, the recent emergence of CWD in the Netherlands is thought to be caused by the violation of the species barrier by the Scrapie TSE, transmitted by contact between infected sheep and free-range reindeer populations [3].

Species Barrier Violations by TSEs

This violation of the species barrier is not exclusive to the Scrapie variant of TSEs presenting further containment issues with the containment of CWD infections. In 1996 beef obtained from cows infected with Bovine Spongiform Encephalopathy (BSE) that were not currently displaying the clinical symptoms associated with TSEs was widely distributed the UK. After a variable latency period of approximately 10 years patients who had consumed the tainted meat began to show symptoms of a variant form of Creutzfeldt-Jakob Disease (vCJD) and were eventually diagnosed with vCJD. As of 22006 there were 200 cases reported worldwide. This

caused the United States to implement a ban on feeding meat and bone meal to cattle intended for U.S. consumption to help stop the spread of BSE among livestock [4]. BSE mutated prions PrP^{BSE} are shown to pass to a range of species, successfully inducing various and potentially novel forms of TSEs. Transmission to other species through a vector species has been theorized. Collinge set out to study the possibility of this hypothesis by intentionally infecting potential vector species that contains its own distinct gene encoding PrP through transcranial inoculation with PrP^{BSE} infected brain matter. It was noted that the transmission characteristics of the PrP^{BSE} remained the same between the induced vector and the target organisms [8]. While there has been no confirmed transfer of CWD to humans unlike what has been seen with BSE induced vCJD, there have been reported cases of persons developing vCJD who self-reported consuming venison at some point in their life. Due to the nature of this reporting this does not indicate causation, only correlation. Additionally, in vivo studies conducted by Jong, Madsen, Rheede, and Smolenaars where macaques, a closer relative to humans, were fed venison or elk meat and brain matter contaminated with PrP^{CWD} or received injections of PrP^{CWD} in their cerebrospinal fluid. The introduced PrP^{CWD} proteins successfully induced the transformation of endogenous PrP^{C} to the formation observed in PrP^{CWD}, indicating the potential for CWD to be transmitted to a more distantly related mammal [1]. Even though transmission to humans is unlikely and unproven the promiscuous nature of all TSEs indicates the potential for transmission of CWD to other wild mammals or livestock kept in close-proximity to infected cervids.

This promiscuity of TSEs is due to the high level of genetic conservation among the different variants. Jong et. al. amplified DNA coding regions for mature prion proteins of 26 eutherian species. A high level of conservation of the protein structure among all PrP molecules studied was observed and DNA sequencing identified a similarly high level of conservation for

the respective PRNP genes of each species was observed [7]. Additional sequencing carried out by Collinge corroborated these findings, illustrating the highly conserved nature of all currently sequenced mammalian PrP genes. This will inevitably lead to a restricted number of possible confirmations of mutated PrP molecules that are both highly stable and thermodynamically permissible [8]. All mutations with pathological significance occur within or adjacent to regions responsible for the protein molecule's secondary structure. This structural change is usually associated with the 2nd or 3rd alpha helix, thus destabilizing PrP structure. These mutations causing the change affect repeat numbers in the PRNP gene. A high enough repeat number is needed for proper copper ion binding but too high promotes early onset of prion disease. The observed conservation of PRNP genes and PrP molecules is thought to be the underlying mechanism responsible for the species barrier violation exhibited by TSEs [7].

Transmission of Chronic Wasting Disease

Chronic Wasting Disease has been shown to be highly infectious and the observed transmission patterns highlight the potential for additional methods of infection outside of vertical transfer and consumption of an infected corpse. By using Bayesian statistical analysis, Ortega and Zabel attempted to determine the exact mode of transmission that was until recently unaccounted for by traditional models of inheritance and transmission [3]. In an analysis of two CWD outbreaks Miller, Thompson Hobbs, and Taverner ran seven competing models to study the best fit model for transmission of CWD amongst captive mule deer. Models portraying indirect transmission through the environment had 3.8 times more data support than models representing transmission as a product of direct interactions between infected/susceptible deer [13]. By allowing for indirect transmission through the environment paired with a high level of

prion stability post introduction to the environment they were able to explain the exponential dispersal patterns observed [3].

Many hypotheses have been made as to the identity of the environmental vector responsible for the indirect transmission patterns observed. Qi, Eckland, Telling, Bartz, and Bartelt-Hunt set out to determine the role of soils as potential culprits for this vector. By homogenizing naturally CWD infected elk brains and introducing this solution to a variety of silica based soils followed by ten cycles of drying and wetting, researchers found that while infectability of the PrP^{CWD} molecules in the soil decreased slightly, infection after inoculation with said molecules was still observed [14]. Additional research of Ortega and Zabel has highlighted the key component of soil responsible for the stability of PrP^{CWD} molecules shed by infected animals in the environment. Montmorillonite (MTE), found in numerous forms of clay soils, has been found to bind to all forms of PrP proteins. This binding of the mutated PrP^{CWD} molecules into a PrP^{CWD}-MTE complex helps to hold the molecules stable for extended periods of time, even when exposed to natural rinsing and drying cycles like those carried out in the Qi et al. experiment. This PrP^{CWD}-MTE complex increases the infectivity to rates greater than that seen in non-MTE bound PrP^{CWD} molecules. The PrP^{CWD}-MTE complex additionally allows for infection through insufflation of dust containing said complex, significantly increasing the infectability and transmissibility of CWD. When injected intracranially with the PrP^{CWD}-MTE complex, animals were successfully infected with CWD, suggesting that the formation of this complex does not inhibit or interfere with the potential steric interactions thought to be responsible for the induced PrP^{C} conformational change cascade [3,6]. Further analysis of the PrP^{CWD}-MTE complex has revealed that rumen digestion is ineffective at destroying the infectious PrP^{CWD} molecule. In fact, the formation of PrP^{CWD}-MTE increases bioavailability as

well as overall retention of the infectious PrP^{CWD} molecule when consumed orally. As cervids are known to consume soil as approximately 2% of their diet, both deposited on plants and consumed directly from the ground as a source of essential minerals, these findings provide further evidence for the importance of soil in the hypothesized environmental transmission seen in CWD [3]. Additional information on MTE and its common uses is located in Box 1.

Ortega and Zabel additionally studied the influence of other fomites such as water and plants as potential environmental vectors for CWD. They noted that the resilient PrP^{CWD} molecules are found to live in raw sewage with no loss of infectability. The organic matter in sewage and most natural bodies of water was found to shield PrP^{CWD} molecules from degradation. This data suggests that in addition to the PrP^{CWD}-MTE soil complex, infected water supplies are likely responsible for transmission without direct contact with an infected individual. Following this discovery of PrP^{CWD}'s ability to survive in water researchers also studied the role of plants in the environmental transmission of CWD. By spraying homogenized brain matter that had been naturally infected with CWD on plants and subjecting them to repeated rinsing and drying, similar to the soil study carried out by Qi et al., researchers determined that the PrP^{CWD} proteins bound to plant tissues with alarming efficacy. PrP^{CWD} levels on plant tissues remained at stable levels and infectivity rates consistent with unbound PrP^{CWD}. After analysis of a variety of wheat plants grown in soil mediums containing PrP^{CWD} molecules, significant amounts of the PrP^{CWD} molecules were detected in the roots, leaves and stems. This finding is essential to understanding the mechanisms of environmental transmission as anywhere from 4-64% of mule deer's springtime diet is comprised of barley and wheat grasses like those used in the study [3].

Researchers at the NIAID Rocky Mountain Laboratories set out to determine the minimal amount of infectious material needed to induce infection with CWD. The findings of this research were astounding, identifying that an animal need only ingest approximately five to ten mutated PrP^{CWD} molecules to successfully become infected, setting the LD_{50} value for CWD [4]. In analysis of the shedding rate of PrP^{CWD} exhibited by infected individuals, it has been discovered that an individual infected cervid can shed approximately 1,000 LD_{50} units daily, meaning that one animal sheds enough PrP^{CWD} molecules to successfully infect 1,000 individuals per day. While the spread of CWD is not this high, when paired with the environmental transmission capabilities of PrP^{CWD} molecules, this shedding creates high potential for infection of large herds of cervids, especially in wintertime when herds do not disperse [3,14]. Additionally due to human development and increased land usage, cervid habitats are rapidly shrinking and forcing individuals into closer contact and increasing the transmission potential of CWD [15]. This finding has led to currently ongoing research of the hypothesis that the smaller PrP^{CWD} molecule aggregates are significantly more infectious than their larger counterparts [4]. These findings explain the high rate of transmission of CWD seen in cervids, as well as their unique distribution patterns.

Potential Solutions and Intervention Strategies

Mutated PrP molecules are incredibly difficult to destroy, creating immense problems for CWD control and prevention methods. PrP^{CWD} molecules have been found to adhere to stainless steel and persist despite standard autoclaving sterilization methods [8]. Additionally, these misfolded proteins have been recorded to withstand temperatures up to 600°C without denaturing, presenting additional decontamination complications. Currently the leading theory of how to remove PrP^{CWD} from contaminated water supplies and soil mixtures lies in utilization

of the soil compound responsible for the high level of environmental transmission seen in CWD. By exploiting PrP^{CWD} molecules' high level of affinity for MTE there is great potential for using MTE as a "magnet" to effectively bind to and remove the PrP^{CWD} molecules [3].

While no treatments for infected individuals exist at this time, current testing of an experimental drug PLX5622 that kills microglial cells in the brain in vivo has allowed researchers to study the role of microglia in preventing onset of CWD symptoms. Those treated with the drug displayed a shorter lifespan by approximately one month, indicating microglia's role in preventing symptom onset. This raises additional questions and has provided a launch point for additional research in potential microglia-based treatments for infected individuals [16]. In addition, RML researchers have discovered the presence of a membrane anchor essential for infection with TSEs. By utilizing genetic knockout animals, removing the membrane anchor does not stop formation of the misfolded PrP molecule, but the formation of amyloid plaques and the expression of resulting symptoms was halted. While this presents researchers with a unique approach to developing a pharmacological treatment for CWD and other TSE infected individuals, additional side effects of elevated PrP^{CWD} concentrations are exhibited, primarily deposition in heart tissue and subsequent malfunction [4].

Until 2014 the only way to confirm the presence of CWD or other TSEs was through autopsy, creating problems identifying potentially infected individuals and removing the potential for culling to reduce transmission especially in captive populations. Thankfully due to the discovery of methods to obtain olfactory neurons while the individual is still alive, researchers are now able to screen for PrP^{CWD} and other malformed prions through real-time quaking-induced conversion (RT-QuIC). While cerebrospinal fluid can be tested as well,

utilizing olfactory neurons allows for a faster, less painful alternative, with proven success in agricultural and captive herd research settings [17].

Concluding Thoughts

Chronic Wasting Disease represents a highly prevalent subtype of TSEs that exhibits impressive rates of transmission. While this disease does not present a direct threat to humans it has lasting implications for wildlife populations including but not limited to cervids. Additional research on the mode of transmission is necessary as the current hypothesis negates one of the cornerstones of biology, transmitting disease and inducing changes in vivo without any nucleic acid molecules. Due to the recent increased understanding of the role of environmental transmission in the transmission and dispersal of CWD researchers are left to identify ways to effectively degrade these PrP^{CWD} molecules in the environment to cease further spread.

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Table 1. The different forms of transmissible spongiform encephalopathies

Human Prion Diseases	Animal Prion Diseases
Creutzfeldt-Jakob Disease (CJD)	Bovine Spongiform Encephalopathy (BSE)
Variant Creutzfeldt-Jakob Disease (vCJD)	Chronic Wasting Disease (CWD)
Gerstmann-Straussler-Scheinker Syndrome	Scrapie
Fetal Familial Insomnia	Transmissible Mink Encephalopathy
Kuru	Feline Spongiform Encephalopathy
	Ungulate Spongiform Encephalopathy

Figure 1. A map comparing current distribution of cases of CWD both in both free ranging and captive populations in the United States and Canada with the distribution seen in 2000.

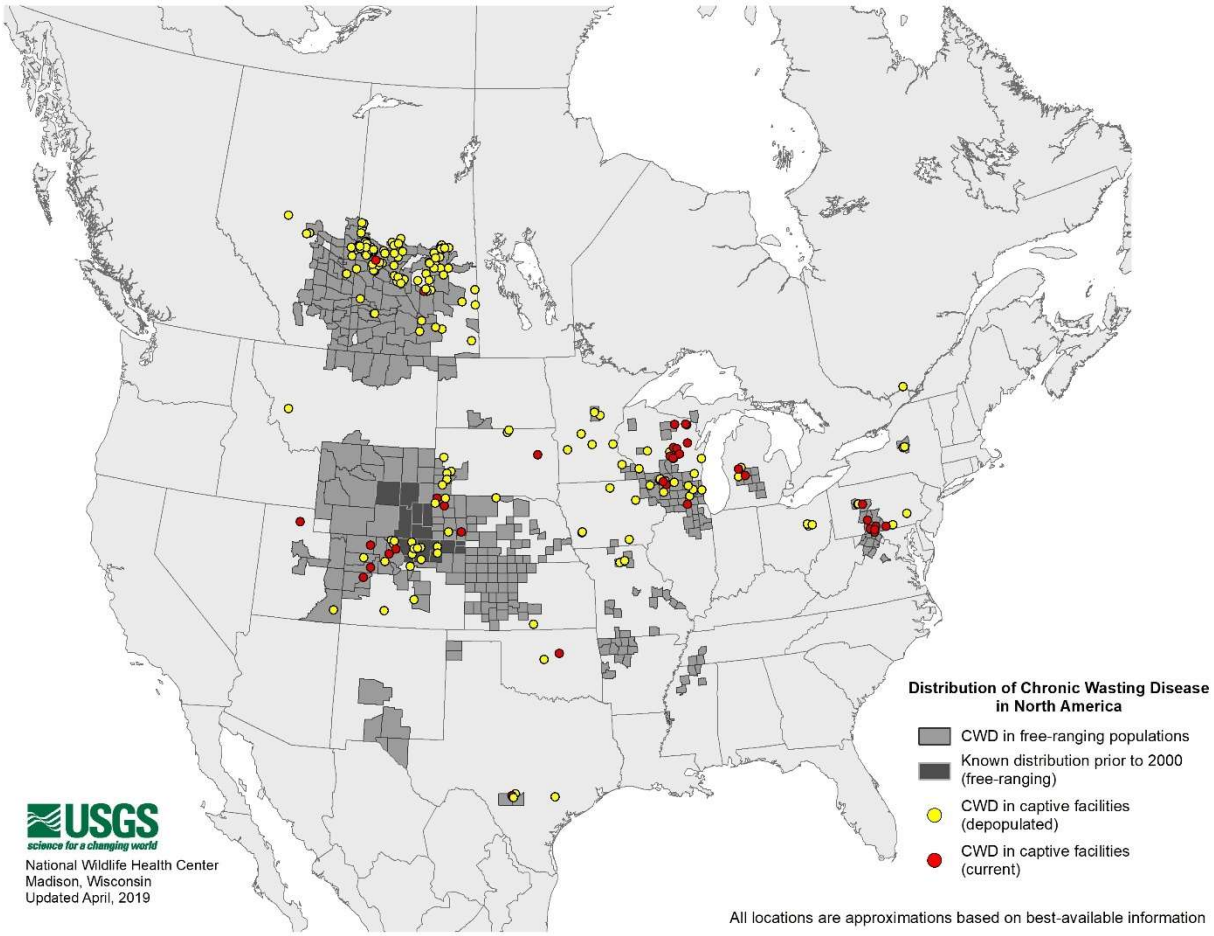

Figure 1: Cases of CWD found in both free range and captive populations of cervids as of April 2019. Areas shaded in dark gray indicate the known free-range distribution of CWD prior to

2000, where light gray areas represent counties where CWD is currently present in free range populations. Yellow dots indicate the frequency of CWD in depopulated captive facilities while red indicates CWD cases currently in captive facilities. Map obtained from USGS, the sole science agency for the United States Department of the Interior. *https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0*.

Box 1. Montmorillonite's Common Uses

Montmorillonite (MTE) has a number of common industrial and agricultural uses, in addition to its natural presence in numerous clay-based soils. The following uses of MTE increase the amounts of environmental MTE and may possibly be responsible for some of the PrP^{CWD}-MTE complex mediated spread of CWD:

- Soil additive for dry and arid landscapes due to its high affinity for CO_2.
- Anti-caking agent in animal feeds.
- Annual well caps.
- Drilling mud used as a lubricant in the oil drilling industry.
- Protective liners for landfills.
- Retention and drainage aid.
- Flocculant in ponds.
- Non-explosive agent for demolishing concrete structures or for splitting rock in natural stone quarries where use of explosives is unadvised.
- Construction of earthen dams and levees.

PubChem (n.d.) Montmorillonite. NIH. Retrieved from: https://pubchem.ncbi.nlm.nih.gov/compound/71586775

Glosarry

- **Ataxia** – loss of coordination of voluntary muscle movements
- **Cervids** – any member of the Cervidae family: deer, caribou, elk, and moose, characterized by the presence of antlers in either males only or both sexes.
- **Fomites** – any agent that is capable of absorbing and transmitting the infectious agent of a disease.
- **Hypotonia** – abnormally low muscle tone.
- **Polydipsia** – abnormally great thirst, resulting from a disease of physiological ailment.
- **Polyuria** – abnormally excessive production and/or passage of urine.
- **Proteolysis** – enzymatic breakdown of proteins or other peptides into their constituent amino acids.
- **Rumen** – the first stomach of a ruminant organism, responsible for fermentation and partial digestion of plant matter.
- **Species Barrier** – natural mechanisms that prevent diseases and viruses from spreading to different species.
- **Torpor** – a state of decreased activity usually accompanied by a reduced body temperature and resting metabolic rate.
- **Wasting** – progressive muscle atrophy.