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REVIEW

Chronic Wasting Disease In Cervids: Prevalence, Impact And Management Strategies

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Abstract: Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) that affects members of the cervidae family. The infectious agent is a misfolded isoform (PrP^{SC}) of the host prion protein (PrP^{C}). The replication of PrP^{SC} initiates a cascade of developmental changes that spread from cell to cell, individual to individual, and that for some TSEs, has crossed the species barrier. CWD can be transmitted horizontally and vertically, and it is the only TSE that affects free-ranging wildlife. While other TSEs are under control and even declining, infection rates of CWD continue to grow and the disease distribution continues to expand in North America and around the world. Since the first reported case in 1967, CWD has spread infecting captive and free-ranging cervids in 26 states in the US, 3 Canadian provinces, 3 European countries and has been found in captive cervids in South Korea. CWD causes considerable ecologic, economic and sociologic impact, as this is a 100% fatal highly contagious infectious disease, with no treatment or cure available. Because some TSEs have crossed the species barrier, the zoonotic potential of CWD is a concern for human health and continues to be investigated. Here we review the characteristics of the CWD prion protein, mechanisms of transmission and the role of genetics. We discuss the characteristics that contribute to prevalence and distribution. We also discuss the impact of CWD and review the management strategies that have been used to prevent and control the spread of CWD.

Keywords: CWD, prion, PRNP, PrP^{C}, PrP^{SC}, TSE

Introduction

Background

Chronic wasting disease (CWD) is the prion disease of the cervidae family.[1] Prion diseases—or transmissible spongiform encephalopathies (TSEs)—are a group of progressive neurodegenerative disorders that affect animals and humans. The first TSE was discovered in the 18th century; at the time it was a strange disease that affected sheep, causing behavioral changes inducing excessive licking, scratching and altered gait.[2] After Scrapie was first described in 1732, other diseases with similar neurological characteristic, such as Creutzfeldt-Jakob disease in 1920 (CJD)[3,4] and Kuru[5] in 1957 were identified in humans.

The agent causing these diseases was not clearly defined but was presumed to be a viral infection of the central nervous system.[6–9] By the year 1959, researchers had linked Scrapie, Kuru and CJD by suggesting that they were related neuropathies.[10,11] Eight more years passed before researchers considered that Scrapie was caused by a proteinase agent.[12–14] By the same year, 1967, a new disease named CWD was

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discovered in a farmed mule deer (*Odocoileus hemionus hemionus*) in Colorado and later in mule deer and black-tailed deer (*Odocoileus hemionus columbianus*) in Colorado and Wyoming, USA;[1] yet, the term TSE was far from being used as a disease category.[2]

It was not until 1982 when Prusiner used the term "prion"—derived from the words proteinaceous and infectious—to describe the causative infectious agent of Scrapie.[15] The same year Prusiner and collaborators proved that the causative agent of Scrapie was a protein. In 1997, Prusiner and collaborators won the Nobel Prize for the discovery of "Prions – a new biological principle of infection" and for their contribution on prion research development. While the consensus is that prion proteins (denoted PrP^{SC} from Scrapie) are the causative agents of prion diseases, and further evidence support that *PRNP*—a host gene that regulates the expression of the prion protein PrP^{C}—plays a crucial role in the development of TSEs,[16] some researchers proposed bacterias[17,18] and viruses[19] as causative agents of TSEs. However, these theories were soon dismissed.[20–22]

Even though the characteristics of the TSEs disease group are clear (Figure 1), they may present as inherited, infectious or sporadic disorders in a variety of hosts depending on the TSE (Table 1).[23,24] Most TSEs are under control or declining. However, CWD is on the rise and is the only prion disease of wild free-ranging animals;[25] CWD continues to affect several cervidae host species across the world. This review will introduce the unique characteristics of CWD and the influence of genetics. We will focus on prevalence and distribution, and examine the impact of CWD and suggested management strategies.

CWD Characteristics

Prion proteins (PrP^{C}) are cell-surface glycoproteins with predominantly α-helical conformations. PrP^{C} is encoded by the prion protein gene (*PRNP*), which is present in almost, if not all, mammalian species. The PrP^{C} are expressed in several tissues and cell types,[26] including epithelial, endothelial and immune cells.[27–30] Above all, PrP^{C} is highly expressed in neurons and neuroglial cells of the peripheral nervous system (PNS) and central nervous system (CNS).[31,32] The infectious prion protein is the misfolded isoform (PrP^{SC}) of the cellular PrP^{C}. The posttranslational process that causes conformational changes from a predominantly α-helical isoform and a coil structure to a refolded β-pleated sheet[33] confers resistance to proteases (eg, environmental, intestinal and intracellular) that would

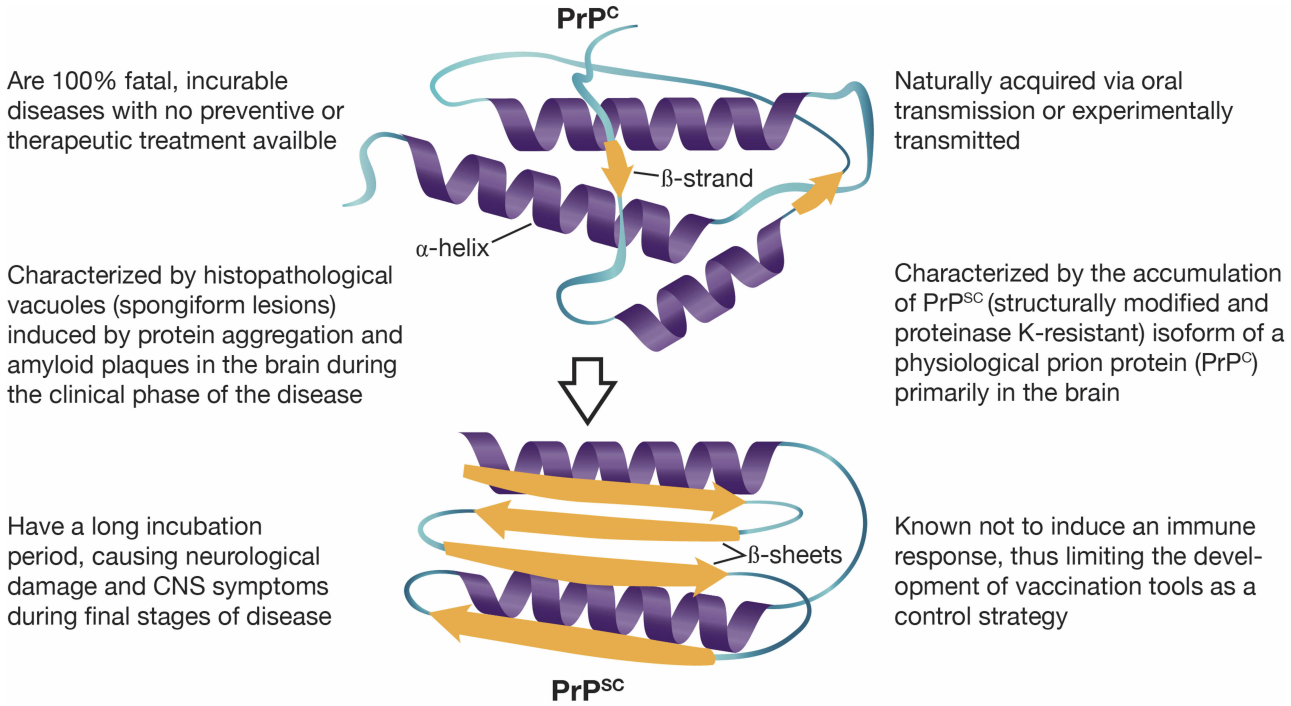

Figure 1 Characteristics of transmissible spongiform encephalopathies (TSEs) or prion diseases.
Notes: Conformational changes of the host prion protein structure, from α-helices in the normal cell-surface glycoprotein (PrP^{C}) to β-sheets in the misfolded isoform (PrP^{SC}). Data from Doherr (2007),[23] Prusiner (1998),[26] Novakofski et al (2005),[34] Image credit to Kerry L. Helms, Scientific Illustrator (Public domain).[159]
Abbreviations: PrP^{C}, the host prion protein; PrP^{SC}, the misfolded isoform of the host prion protein.

Table 1 Human And Animal Prion Diseases

Mammalian Species	Prion Disease Name	First Reported	Affected Mammals Since First Reported	Mode Of Natural Transmission	References
Cervidae	Chronic Wasting Disease (CWD)	1967	North American elk or Wapiti, mule deer, white-tailed deer, moose and Caribou. European red deer, sika deer, reindeer, axis deer, roe deer, fallow deer, Muntaj and moose	Horizontal transmission via direct contact with CWD infected cervids or indirectly via contact with CWD contaminated plants or inanimate fomites (eg, Soil, mineral licks, plastic reservoirs, etc). Vertical transmission from mother to offspring in utero	38, 48, 101, 113, 114, 154, 155
Bovidae	Scrapie Bovine Spongiform Encephalopathy (BSE)	1732 1986	Sheep, goats Cattle	Horizontal and vertical (same as CWD) Exposure to contaminated TSE tissue in food (MBM)	2, 26, 154
Hominidae	Sporadic Creutzfeldt-Jakob Disease (SCJD)	1920	Humans	Unknown etiology (occurs spontaneously)	2, 3, 4, 5, 26, 154
	Familial Creutzfeldt-Jakob Disease	1924	Humans	Familial prion diseases (inherited)	
	Gerstmann-Straussler-Scheinker Syndrome (GSS)	1936	Humans	Familial prion diseases (inherited)	
	Kuru	1957	Humans	Exposure to prion contaminated tissue (brain) during cannibalistic funeral rituals	
	Iatrogenic Creutzfeldt-Jakob Disease (ICJD)	1974	Humans	Exposure during surgery to CJD-contaminated instruments or via organ and tissue transplant	
	Fatal Familial Insomnia (FFI)	1986	Humans	Familial prion diseases (inherited)	
	Variant Creutzfeldt-Jakob Disease (VCJD)	1996	Humans	Exposure via ingestion of BSE-contaminated food	
	Sporadic Fatal Insomnia (SFI)	1997	Humans	Unknown etiology (occurs spontaneously)	
Mustelidae	Transmissible Mink Encephalopathy (TME)	1965	Mink	Unknown etiology (occurs spontaneously)	26, 154
Zoo Animals	Ungulate Spongiform Encephalopathy	1988	In zoo animals including greater kudu, Arabian and scimitar oryx, nyala, the common eland, Ankole-Watusi cow, gemsbok, and American bison.	Exposure via ingestion of BSE-contaminated food (MBM)	156, 157
Felidae	Feline Spongiform Encephalopathy (FSE)	1990	Housecats and captive wild cats including tigers, pumas, lions, cheetah, ocelot, Asian golden cat and Asian leopard cat	Identified in domesticated and captive wild cats exposed to BSE-contaminated food (bovine tissue or meat and MBM)	156, 157
Camelidae	Camel Prion Disease (CPD)	2015	Dromedary camels	Unknown etiology	158

Abbreviation: MBM, meat and bone meal.

otherwise destroy the protein.[34] Besides resistance, the β-sheet structure of the pathogenic PrP^{SC} is prone to aggregation. Aggregation of PrP^{SC} leads to the conversion of more PrP^{C} to PrP^{SC}, formation of amyloid plaques and vacuolization that cause progressive neurodegeneration.[35] Because the modification from PrP^{C} to PrP^{SC} is posttranslational, the amino acid sequence of both, PrP^{C} and PrP^{SC} (209 residues), is identical within an individual.[24] Hence, there is not a substantial immune response and inflammatory reaction to the infection.[36,37] However, chronic inflammation may contribute to natural CWD transmission, as chronic inflammation may upregulate cytokines enabling PrP^{SC} accumulation and propagation to other tissues.[34] For example, follicular dendritic cells and mast cells express high levels of PrP^{C}.[28,36] Expression and release of PrP^{C} from migratory cells, such as mast cells, may facilitate quick progress of the infectious prion from lymphoid tissues associated with the gastrointestinal track to the PNS, CNS and brain.[28]

Prions are infectious pathogens that, in the case of CWD, can be transmitted horizontally or vertically. Horizontal transmission is the most effective CWD transmission method, with reported incidence of disease in captive mule deer of 89%,[38] and early infection detected in lymphoid tissue along the oral and digestive system 42 days post-oral inoculation in mule deer fawns.[39] Horizontal transmission includes direct contact of an infected and susceptible animal or contact of susceptible animals with infected saliva, feces and urine. Indirect horizontal transmission involves environmental components and includes oral infection by ingestion of contaminated grass and/or soil during grazing and dust inhalation of infectious particles bound to soil.[40,41] Based on social networks and contact patterns among free-ranging white-tailed deer (*Odocoileus virginianus*), direct contact is the primary contributor of CWD transmission among deer.[42]

Scientists considered vertical transmission (in utero) an unexpected or rare process. Researchers believed that high neonatal mortality in deer and elk populations coupled with the solitary nature of cervids during parturition reduced the importance of maternal transmission in sustaining CWD.[43] However, TSEs vertical transmission has been confirmed in sheep, cattle, felids, humans and in transgenic mouse models.[44–47] Recent studies based on experimental models of CWD demonstrated the transmission of CWD from doe (clinical and sub-clinical mothers) to fawns (full-term viable, full-term non-viable and in utero harvested offspring).[48] These studies found 80% of the fetuses from CWD-positive muntjac deer dams PrP^{SC} positive, suggesting previous underestimation of the transmission from mother to offspring for all TSEs.[48] Vertical transmission contributes to CWD infection in naturally exposed elk populations.[49]

The development of clinical CWD can take months to years. The incubation time—the period between exposure to the pathogenic CWD prion to the development of clinical signs and symptoms—in both, naturally and experimentally infected cervids may vary from 2 to 4 years.[43] Differences in incubation periods could relate to infectious dose, route of exposure, cervid species and/or genotype. For example, incubation periods in orally inoculated mule deer ranged from 3 months to 2 years, with differences in CNS accumulation timing associated to genotype profiles.[50] Similar findings were reported for other cervid species; CWD-positive muntjac deer developed terminal disease in 18–24 months post-oral inoculation.[69] Interestingly, viable offsprings from those CWD-positive muntjac dams surpassed the time usually seen for terminal disease in cervid species (18–24 months).[48] Although the maximum incubation period in free-ranging cervids is unknown, most CWD cases have been reported in 3- to 7-year-old animals,[43,51] which is similar to the age groups of captive elk and mule deer that succumb to CWD.[1] Because the way prions spread throughout the body, pathological changes and distribution of PrP^{SC} might be first identified in the lymphoreticular system and later in the CNS, similar to what has been described for sheep infected with scrapie.[52] Retropharyngeal lymph node (RLN) and medulla oblongata at the level of the obex are early sites of PrP^{SC} accumulation[50] and considered gold standard tissues for postmortem CWD detection using immunohistochemistry (IHC). Peripheral accumulation and the excretion of the infective prion protein have been thought to occur only after central nervous system replication and was associated with the time of clinical disease manifestation.[53] However, recent findings identified shedding in excreta concurrent with peripheral lymphoid accumulation.[54] Newer antemortem detection methods such as serial protein misfolding cyclic amplification (sPMCA) and real-time quaking-induced conversion (RT-QuIC) are emerging as potential tools to detect low levels of PrP^{SC} in excreta and identify early accumulation of PrP^{SC} in peripheral tissue of sub-clinical CWD cases.[49,55]

A slow wasting process that leads to death characterizes CWD. Clinical signs—objective evidence of disease—include polydipsia and polyuria (excessive thirst or urination), sialorrhea (drooling or excessive salivation)

and wasting (drastic weight loss). Behavioral changes include listlessness, aggression, lack of fear of people and depression.[53] Signs of neurological damage at later stages are characterized by a lack of coordination, difficulty moving and ataxia (losing balance while walking). Other distinctive characteristics associated with CWD are drooping head and ears. The development of signs and symptoms—subjective evidence of disease—is progressive, with some of them such as polyuria and sialorrhea, appearing at later stages of disease and contributing to the shedding of the pathogenic PrP^{SC}. Diagnosis based on clinical signs and behavioral changes is not possible, as these can be characteristics of other diseases.

Genetics

Eliminating or controlling the spread of TSEs has relied heavily on a solid understanding of the molecular mechanisms of the disease. Earlier work demonstrated that a pathogenic prion protein (PrP^{Sc}) is responsible for post-translational conversion of the host encoded cellular prion protein (PrP^{C}) in several TSEs.[16,24,56] The *PRNP* gene which encodes the PrP protein is well conserved among mammals,[57] including cervids, and has implications for CWD.[58] Though other loci were examined for their involvement (such as "*Sinc*"[59] and *Pid-1*[60]), variations in the prion protein (*PRNP*) gene have been shown to affect TSE progression[61] and susceptibility.[62–64] Due to this association, much of the research on the genetics of CWD has focused on this locus within affected cervids.

Complete genetic resistance has not yet been found for cervids, though examination of *PRNP* sequences has identified variable sites that may influence an individual animal's susceptibility to or the rate of progression of CWD. The inferred amino acid sequence was described in Rocky Mountain elk (*Cervus elaphus nelsoni*), finding only a single mutation of methionine (M) to leucine (L) at cervid codon 132 with elk homozygous (M/M) overrepresented among those infected with CWD.[65] Later studies demonstrated that the L mutation causes an increase in the incubation times.[66] Few studies have been published involving moose (*Alces alces*) and reindeer (*Rangifer tarandus*) due to the rarity of naturally occurring cases of CWD in the wild.[67] Among moose variable sites have been identified at codon 36 (N-asparagine or T-threonine),[68] 109 (K-lysine or Q-glutamine), 90 and 209 (M-methionine or I-isoleucine);[69,70] however, it is unclear what protective qualities these mutations may or may not have for CWD infection. Mule deer and white-tailed deer have been studied extensively, likely due to the higher prevalence of CWD among these species.

Studies of the *PRNP* gene in mule deer and white-tailed deer have identified a number of mutations both in the amino acid and nucleotide sequences. Initial studies were complicated by an unexpressed process pseudogene in some but not all individuals.[71] Further study of the pseudogene did not reveal any effects on CWD infection in deer and the presence of an asparagine mutation at codon 138 is absent in the functional gene, thereby easily distinguishing the two.[72] The functional *PRNP* gene has been studied extensively, notably two coding mutations have been identified in the inferred amino acid sequence that has been linked to reduced CWD susceptibility. Examination of allele frequencies found few CWD-infected individuals with a substitution of histidine (H) for glutamine (Q) at codon 95 (aaQ95H) or a substitution of serine (S) for guanine (G) at codon 96 (aaG96S).[72,73] The effects of these mutations were examined experimentally by orally infecting captive white-tailed deer with known genotypes finding that these mutations delay onset of CWD. Deer genotypes in this study included wild type (aa95QQ/aa96GG, N=6), heterozygous at aa95 only (aa95QH/aa96GG, N=1), aa96 only (aa95QQ/aa96GS N=4) or heterozygous for both positions (aa95QH/aa96GS, N=1).[74] All deer presented with clinical signs of CWD (with the exception of two deer euthanized due to intercurrent disease); wild type genotypes having an average incubation period of 693 (± 27) days, those with only the aa96 mutations lasting 956 (± 107) days, and those with only the aa95 or both aa95 and 96 mutation genotypes succumbing to the disease after 1508 and 1596 days (respectively).[74]

Examination of complete *PRNP* nucleotide sequence corroborates these previous findings of CWD susceptibility and further the understanding of the role of this gene in disease management. Kelly et al[75] found ten polymorphic sites in the *PRNP* gene from free roaming deer in Illinois (N = 196 deer, 76 CWD-positives and 120 CWD-negative). This study identified both of the nonsynonymous mutations (aa95 or nt285, and aa96 or nt286) confirming previous findings and identified three additional synonymous mutations which were determined to be more common among deer testing negative for CWD. Similarly, Wilson et al[76] examined both white-tailed deer and mule deer in Canada finding fifteen variable sites among white-tailed deer and two variable sites in mule deer. Of these variable sites, only one nonsynonymous mutation (nt286) and four synonymous mutations were determined to be

associated with CWD susceptibility in white-tailed deer,[76] and one nonsynonymous (nt59) and one synonymous (nt393) mutation was identified in mule deer, each found significantly more often in mule deer testing negative for CWD.[76]

When the combined effects of synonymous and nonsynonymous mutations were considered from white-tailed deer in Illinois and Wisconsin twenty-six unique haplotypes were identified consisting of fourteen polymorphic sites (ten previously reported[75,76] and four novel).[77,78] Two haplotypes designated C and F were found less frequently among deer testing positive for CWD. Each haplotype contains one of the nonsynonymous mutations reported to reduce CWD susceptibility as well as one synonymous mutation. Haplotype C includes the nonsynonymous mutation nt286A (aa96S) and one synonymous at nt555T, and haplotype F contains the nonsynonymous mutation nt285C (aa95H) and one synonymous at nt60T. Unlike scrapie, no study has identified mutations that confer complete genetic resistance to CWD. Brandt et al[77] found that deer with either the C or F haplotypes were less likely to be infected with CWD but still detected positive deer possessing these haplotypes.[77,78] No studies have examined the protective effects of these mutations with regard to infectious dose of the prion protein.

The absence of complete genetic resistance to CWD does not preclude the use of genetics as a tool to manage the disease. Several studies analyzing white-tailed deer landscape and population genetics in Wisconsin and Illinois have led to a better understanding of deer movement patterns and other dynamics that may influence the spread of CWD. Deer were genotyped using microsatellite loci finding that population structure was largely influenced by female philopatry[79] and landscape features can promote or inhibit movement thus influencing disease spread.[80–82] The frequency of protective *PRNP* haplotypes may contribute to population level susceptibility and shape the way CWD spreads across the landscape. In Illinois where populations have higher frequencies of the protective C or F haplotypes, the geographic progression of the disease was slowed and confined to a smaller area.[78] Control of CWD may require a multifactorial approach where genetic profiles can assist in the management of CWD.

Prevalence And Distribution

The origin of TSEs, and thus CWD, is not clear. Speculative theories suggest that TSEs might have a spontaneous origin, however, these theories are not proven.[83,84] Prevalence of CWD varies across North America, reaching 30% for free-ranging populations in endemic areas,[53] but can be, in unusual circumstances, as high as 80–90% in captive populations.[54] Initial endemic zones were limited to northeastern Colorado and southeastern Wyoming, with eventual growth to southeastern Wisconsin, extending east to New York and West Virginia, and southward to New Mexico.[85,86] Since the first report in captive mule deer in Colorado nearly 50 years ago,[1,54] CWD in North America has spread to 26 US states and three provinces in Canada (Saskatchewan, Alberta and Quebec; Figure 2).[87] The first cases of CWD outside North America were reported by the year 2000 in South Korea, after import of subclinical CWD infected farmed elk from Canada.[88,89] Recently, CWD cases have been found in free-ranging reindeer and moose in Norway, Finland and Sweden.[67,87,90] CWD's expanding geographic distribution has been attributed to both natural movements of free-ranging cervids, as well as anthropogenic movement of infected farmed elk and deer. Movement of animal carcasses and other animal byproducts that are known to be infectious under experimental conditions—including natural cervid urine lures and antler velvet—may be involved in facilitating the spread of CWD.[25,91]

Since the initial report of CWD in mule deer (*Odocoileus hemionus*) in a Colorado research facility in the late 1960s,[1] many captive and free-ranging cervid populations have been affected, and, by the time of writing this paper, the known host of CWD has grown to include moose (*Alces alces*), North American elk (*Cervus canadensis* and *Cervus elaphus elaphus*, also known as wapiti), white-tailed deer (*Odocoileus Virginianus*), red deer (*Cervus elaphus*), sika deer (*Cervus nipon*), reindeer (*Rangifer tarandus*), European moose (*E. alces alces*, also known as Eurasian Elk), mule deer (*Odocoileus hemionus*) and subspecies black-tailed deer (*O. hemionus columbianus*; Figure 3).[92] Although the spread of CWD is well understood, no conclusive evidence to demonstrate a link between CWD in North America and CWD in European cervids has been established.[67] This is mainly due to the lack of understanding of the origins of CWD coupled with no evidence of CWD in the cervid population in the European Union prior to 2016,[93] when the first case of CWD in Europe was discovered in a free-ranging Norwegian reindeer.[91] Still, prevalence of CWD in North America, Europe and possibly other parts of the world is unknown.

Prevalence estimates are susceptible to the number of deer tested, representing the occurrence of disease in the

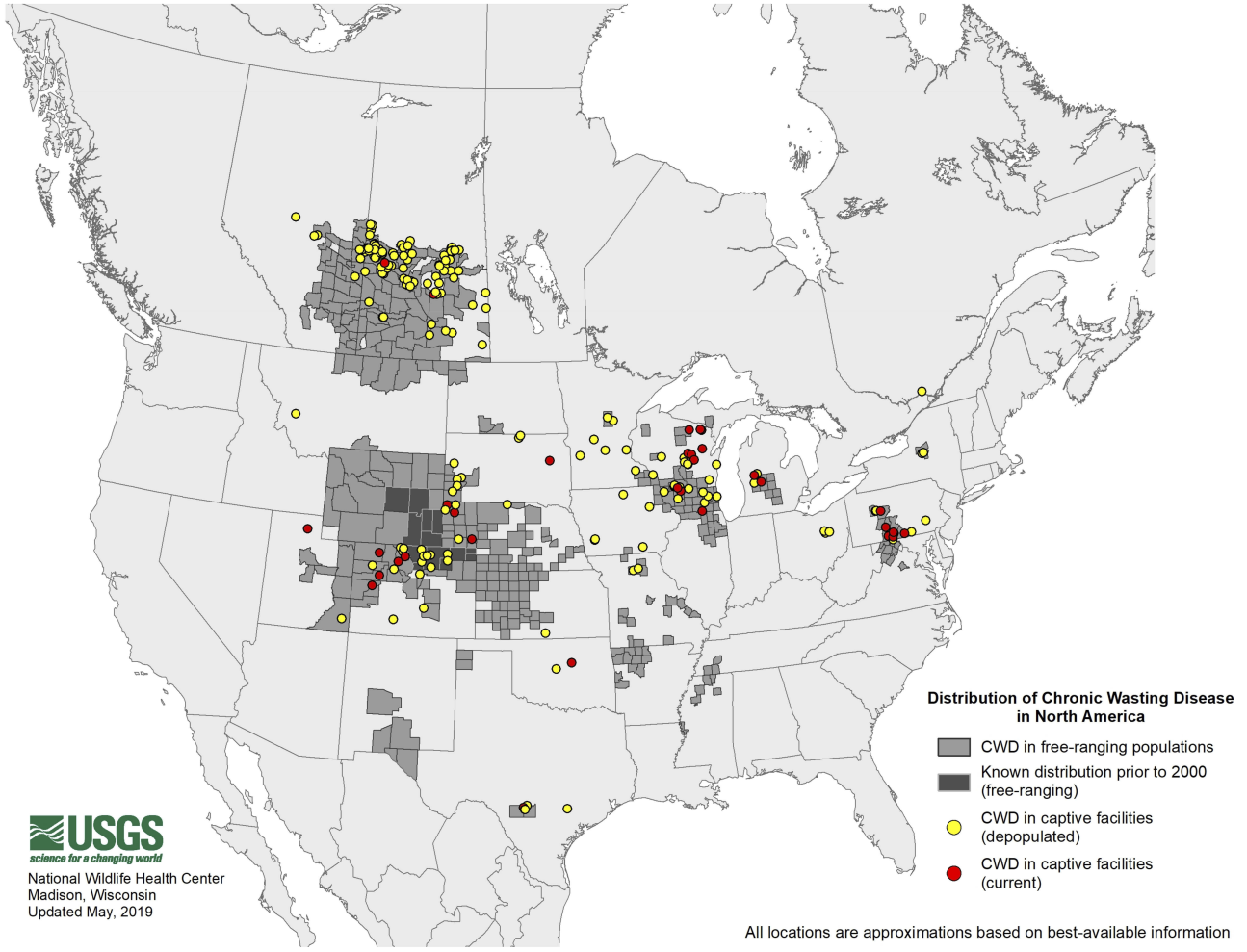

Figure 2 Reported distribution of Chronic Wasting Disease (CWD) in North America. By 2019, 26 states and 3 Canadian provinces have reported CWD cases in captive and free-ranging cervid populations.
Notes: Credit to Bryan Richards, USGS National Wildlife Health Center (Public domain).[160]

tested population in a geographic region at a particular time. Based on hunter-harvested animal surveillance programs (1996–1999), CWD prevalence in an endemic area in Colorado was estimated at approximately 5% in mule deer, 2% in white-tailed deer and <1% in elk.[52] By 2018, CWD rates of infection were estimated to occur in about one-third of Colorado's elk population and about half of the state's deer population.[94] In Wisconsin, CWD prevalence in white-tailed deer doubled in some areas during a period of 6 years (2011–2016), with approximately 40–50% adult males and 20–30% adult females infected.[95] Despite reports of increasing rates of CWD in specific locations in the US, surveillance data from other endemic areas indicate that CWD prevalence rates have remained low and changed little over long periods of time.[96] This is the case in Illinois, where surveillance and management strategies were implemented and sustained since the first case of CWD was detected in 2002. Although prevalence rates found in Illinois in 2018 were lower than previous years, it was recognized that it is too early to suggest that this trend will continue. Thus, long-term surveillance and CWD management strategies need to continue to slowdown the spread of disease and the increase in prevalence rates to parts of the state that remain CWD free.[96]

Prevalence is influenced by biotic factors, such as sex and age, as well as abiotic factors associated to geographic location (eg, soil and pH characteristic). Trends in prevalence in endemic areas in Wisconsin have increased during the last 17 years, showing a rise in the prevalence from 8–10% to over 35% in adult males and from 3–4% to over 15% in adult females at the western monitoring area; during the same period of time the trends in prevalence increased from 2% to 13% in male yearlings and from 2% to 10% in yearling females.[97] In Illinois, age and sex have

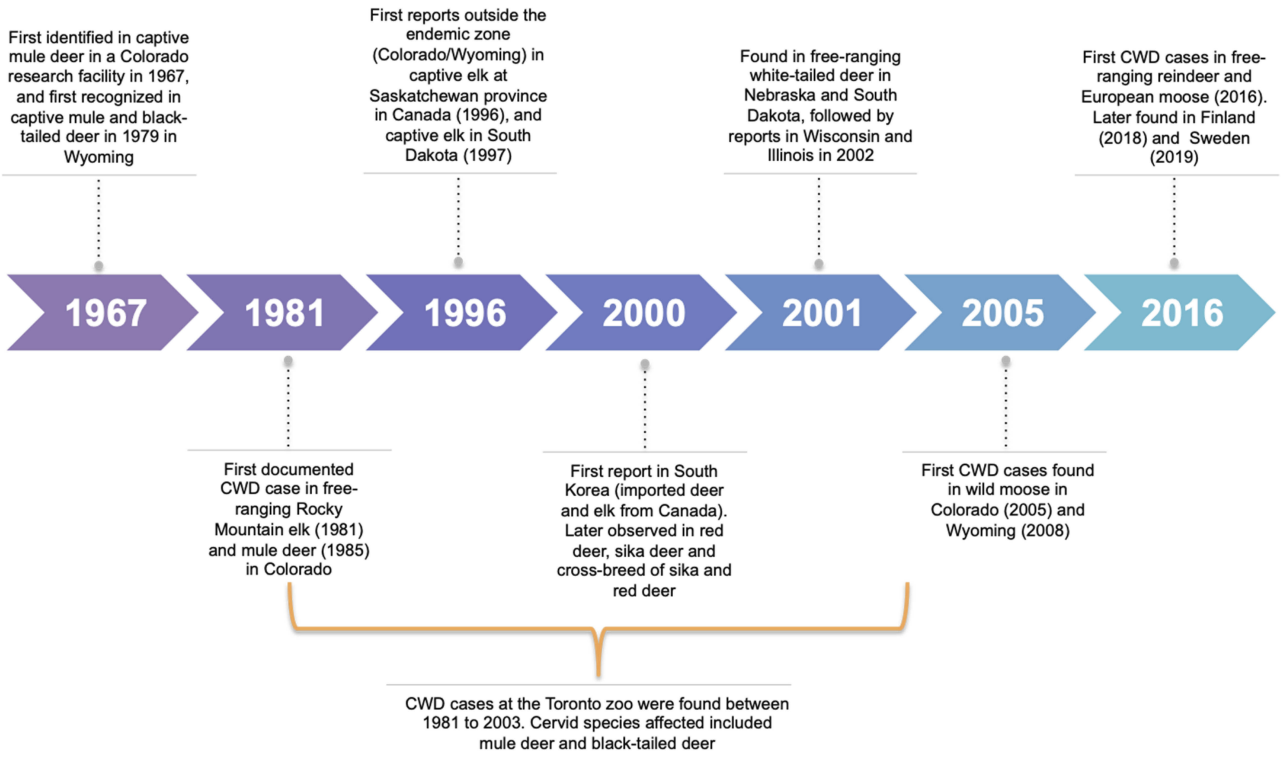

Figure 3 Chronological identification of CWD in cervid species.
Notes: Data from Haley and Hoover (2015),[54] Benestad and Telling (2018),[52] Ricci et al (2017),[67] Chronic Wasting disease Alliance.[92]

been found to be associated with differences in prevalence. The mean prevalence rates during 2003–2018 have been 75% higher in males than in females;[96] with higher rates in adult deer (1.93%) than in yearlings (0.89%) and fawns (0.45%); and higher rates of CWD in males than females, although, in this study, sex difference was not significant (P=0.079).[98] More recently, the overall CWD prevalence in adult deer was estimated at 0.84% and was twice as high in males (1.07%) compared to females (0.54%).[96]

Horizontal transmission is the most important route of CWD infection[52] and the most important contributing factor for CWD prevalence and incidence. Miller et al[41] demonstrated that naïve animals could contract CWD when using sites where previous CWD-infected animals were housed. Prions enter the environment via decaying carcasses and excretion of bodily fluids that have been identified as containing high levels of PrP^{SC}. Blood and saliva are the biological fluids with highest PrP^{SC} levels, followed by fecal matter and urine, thus carrying high levels of infectivity. Other peripheral organs that accumulate large numbers of PrP^{SC} include adrenal glands, thyroid glands, lungs, liver, kidneys, bladder, pancreas, gastrointestinal tract, retina, antler velvet, heart, tongue and skeletal muscle.[52] Prions from all these tissues can enter the environment and remain infectious for long periods of time.[41,99,100] Soils and other fomites acting as environmental reservoirs (eg, mineral licks) contribute to horizontal transmission.[101]

Because of cervid grazing behaviors, infection can be acquired via soil ingestion or soil inhalation, and by contact with bioavailable PrP^{SC} from biological material in soil. It is not surprising that, because of this, much research in recent years has focused on soil properties (eg, organic matter, clay content, soil metals and soil pH) and its contribution to PrP^{SC} persistence in the environment.[98,100,102–109] For instance, attachment of the prion proteins to minerals in clay may limit migration of the infectious CWD protein through the soil column, maintaining infectious PrP^{SC} at the soil surface, contributing to CWD dissemination.[103] While some of these soil characteristics may influence PrP^{SC} stability, persistence in the environment and infectivity,[100,104–106] others—such as natural oxidants and soil humic acids—may interfere with conversion of PrP^{C} to the pathogenic PrP^{SC},[107] or degrade PrP^{SC} and reduce CWD infectivity.[108]

Modeling studies based on CWD cases from surveillance programs have evaluated landscape features related to deer habitat and soil characteristics that could be

environmental determinants for CWD risk, influencing prion availability and persistence.[98,109–111] Evaluation of spatio-temporal patterns of CWD reported cases in white-tailed deer at the border of Wisconsin and Illinois—one of the hot spot areas for CWD in the US—found that landscape features such as larger and more compact forest, as well as lower elevation areas closer to rivers, were associated with higher risk of CWD; yet, other study found areas with small forest patches increased the risk of CWD occurrence.[110] A geographical model focused on soil characteristics and its contribution to CWD in free-ranging deer found percent of clay and soil pH as the two most important predictors of the persistent presence of CWD in endemic areas.[109] This is in agreement with findings by O'Hara-Ruiz et al[98] that indicated that less clay and more sand enhance CWD persistence and transmission. Still, while some studies agree that more clay is associated with less CWD, others have found the opposite[106] or no association to CWD incidence.[112]

Beside soil, other common environmental materials including wood, rocks, plastic, glass, cement, stainless steel, aluminum and grass plants have been proven to "bind, retain and release" prions.[113,114] In the case of plants, these can act as carriers of infection by binding infectious prions from contaminated secretions, as well as by uptake of prions from contaminated soils, and mobilizing them to aerial parts of the plants including steam and leaves.[113] After five decades of CWD research, many factors that influence CWD prevalence have been identified and several lines of evidence have expanded our understanding of how CWD spreads in nature. Nonetheless, many questions remain and significant challenges need to be addressed in order to effectively control CWD prevalence and reduce incidence at different geographic locations.

Impact

Chronic wasting disease has an ecologic, economic and social effect, with deep impact on the viability of cervid populations. An experimental study found a 60% decline in full-term viable offspring born to CWD-positive muntjac dams.[48] Modeling studies have shown an annual population decline of 10.4% in white-tailed deer and 21% in sympatric mule deer populations in southeastern Wyoming, corroborating the population-limiting impact of CWD.[115,116] Survival estimates indicate that CWD-infected mule deer were 4.5 more likely to die annually compared to CWD-negative deer[116] and were more susceptible to predation than uninfected deer.[117] The impact on elk populations in endemic areas in Colorado and South Dakota has also shown declines in survival[118] and decrease in population growth rates.[119] Conversely, the impact of CWD in low-density deer populations differs from places with high-density populations. Mule deer living in arid San Andres Mountains—part of the Chihuahuan Desert-range in southern New Mexico—showed weak population effects based on CWD prevalence and mortality data.[120] Models reveal mixed results in long-term survival of cervid populations based on observed epidemics in endemic areas of Wisconsin, Colorado and Wyoming. Outcomes ranged from small host declines to moderate epidemics, and in some cases, to complete host extinction.[121] Captive cervid facilities have been impacted by CWD, with over 175 herd facilities affected across the US and reported infection rates as high as 80% at some of these facilities.[122] The extent of disease impact in other parts of the world is less understood. Because of the limited surveillance across Europe—especially in remote areas—it is not possible to exclude the possibility that CWD has been affecting cervids across Europe for decades.[91,93]

Another consideration of the potential impact of CWD is the risk to human health. Even though the only demonstrated zoonotic TSE is variant Creutzfeldt–Jakob disease (vCJD), which resulted from non-experimental transmission of classic bovine spongiform encephalopathy (BSE) from cattle to humans,[123] no absolute molecular barrier to conversion of the human prion protein by the CWD prion protein has been found.[124] Experimental studies and epidemiological investigations, coupled with careful surveillance, established a link between vCJD and BSE. Nevertheless, ongoing surveillance and epidemiological studies of humans living in CWD-endemic areas in North America and Canada have not shown any increases in human TSE cases,[123,125] and have not been able to find associations between CWD and prion diseases in humans. Laboratory and epidemiological data support the role of a species barrier protecting humans from CWD.[126–131]

Experimental studies using humanized transgenic mice did not result in CWD transmission,[127] and Raymond et al[126] demonstrated a barrier at the molecular level that appears to limit the susceptibility of humans, cattle and sheep to CWD. Yet, susceptibility to CWD has been shown in cattle, cats, sheep and goats under experimental conditions following intracerebral inoculation.[132] Oral inoculations, on the other hand, have been inefficient at inducing disease, suggesting a high species barrier under oral exposure. Only recently oral inoculation

was successful at inducing prion disease in squirrel monkeys and swine.[130,131,133] In swine, the species barrier was relatively high, as only low amount of prions was found in brain and lymphoid tissue.[133] Interestingly, CWD has not been successfully transmitted to Cynomolgus macaques, which are genetically closer to humans than squirrel monkeys.[129,131,134]

More recently, the zoonotic potential of scrapie prions was demonstrated after serial transmission of different Scrapie isolates to humanized transgenic mice,[135] coupled with the reported transmission of Scrapie prions to primates after long incubation periods of 10 years.[136] Taking into account the long incubation periods—of a minimum of 5 years—that was required before clinical disease was observed after oral inoculation of Cynomolgus macaques with BSE, surveillance and research should continue and allow for long incubation periods to elucidate long-term effect of CWD in nonhuman primates and potential consequences to humans.

Beyond the direct impact of CWD on free-ranging cervid populations and potential effect on human health, there is an economic impact associated with management of CWD, and the effect of CWD on hunters and farmed cervid industry. For example, after the discovery and spread of CWD in North America, an estimated $32.3 million was spent by Wisconsin for CWD surveillance and management between 2001 and 2006.[137] The potential economic losses per farm have been estimated at $290,000,[138] and reached $53–$79 million in 2002 and $45–$72 million in 2003 for hunters in Wisconsin.[139] Total depopulation was required at some captive cervid facilities, with costly government expenses associated with compensation.[95] According to the 2016 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation,[140] an estimated 103 million Americans participated in fishing, hunting or other wildlife-associated recreational activity, spending $156.9 billion on equipment, travel, licenses and fees. Approximately 11.5 million were hunters, meaning that 4% of the Americans 16 years of age or older hunted in 2016. Revenues from hunting, fishing and wildlife-associated activities help to support wildlife and habitat conservation efforts. However, concerns about the potential and long-term impact of CWD to the cervid captive and wildlife populations, compounded with unknown risks of CWD transmission to humans, and evidence of risk of transmission to swine, could impact these revenues. CWD may reduce hunting and related activities in endemic areas, affecting the cost of management disease in areas where CWD becomes established. These, in turn, could affect jobs and communities that depend on the support of hunting and related activities across the nation.

Management Of CWD

Management guidelines for infectious diseases like CWD are difficult to develop and implement, as they need to account for factors that influence prevalence, incidence, transmission and geographic spread. Some of these factors include population dynamics, genetics, animal movement and dispersal, type of population (eg, captive or free-ranging cervids) and landscape characteristics (eg, forest areas or arid environments). Furthermore, the goals of proposed management and control strategies of CWD should be defined according to disease status in different regions; only then, strategies for control and/or prevention might be implemented. While depopulation of an infected herd followed by restocking after a period of 2 years is used for farmed deer,[141] management intervention strategies for free-ranging populations are different; they consist of population reduction—to minimize disease transmission—and selective culling of deer in CWD endemic areas—to control CWD prevalence.[142] These efforts require support from hunters and landowners so that the management can be applied. Eradication of CWD might not be realistic, but control is; once CWD has become established, management strategies should focus on limiting the growth of the number of infected individuals and therefore limit the increase in prevalence.

Farmed cervid CWD management programs in the US have been developed with the goal of creating a national approach to control CWD incidence and prevent spread between states. This is a collaborative effort among state regulatory agencies (eg, wildlife and animal health agencies), Animal and Plant Health Inspection Services (APHIS) and owners of farmed cervid facilities. The USDA-APHIS CWD management program includes a herd certification program that facilitates surveillance and interstate movement of non-infected animals. The program provides guidance on fence design, sampling strategies and response protocols if CWD is detected in a facility (eg, quarantine and carcass disposal, decontamination procedures and management of a herd during the epidemiological investigation).[143] Depending on the epidemiological investigation, a herd could be classified as (a) CWD-positive (if an animal tested positive for CWD), (b) CWD-exposed ("if a CWD-positive animal resided in another herd (or multiple herds) within the previous 5 years") or (c) epidemiologically linked herds (all herds with

animals that were in contact with other animals that previously resided with a CWD-positive animal). If a herd is classified as CWD-positive or CWD-exposed, a quarantine of 5 years should be issued—based on the date the herd was last exposed to a CWD-positive animal—unless the herd is depopulated.[143]

The four CWD management strategies used in North American free-ranging wild deer include 1) general, non-selective population harvest (spatially targeted); 2) selective or targeted removal of clinical suspects (infected deer); 3) seasonal harvest (eg, summer) and 4) vaccination.[144] Predictive models that evaluated these management strategies found that increased general hunting pressure with or without targeted sex group, the role of large predators, and seasonal hunting had some positive effect on CWD under specific conditions.[145–147] Yet, analytical experimental studies that included vaccination (eg, intramuscular vaccination with two different prion peptide sequences) and oral administration of therapeutic compounds for prevention of CWD infection showed ineffective results.[148,149] A study evaluating the use of mucosal immunization with an attenuated *Salmonella* vaccine expressing PrP found that the efficacy of the control measure was not clear.[150] Two analytical observational studies based on planned culling as the intervention strategy found the control measure effective.[144] Despite differences between intervention and control strategies, studies that evaluated differences between government culling and hunting, found that moderate but sustained intensity with continued and frequent culling is needed to reduce CWD prevalence.[142] This effort minimizes the impact on recreational deer harvest.[151] Furthermore, other studies suggested that management strategies focused on reducing population prevalence instead of deer abundance are more effective strategies in reducing CWD transmission.[112]

The objective of management and surveillance is to protect the health of captive and free-ranging herds from the spread of CWD, mitigate the negative consequences of reduced recreational hunting on the economy, decrease the geographic spread of CWD and reduce the potential of CWD prions to be transmitted to the environment, humans and any other species.[98] Surveillance and monitoring of CWD provide essential data that help with the development of focused management strategies in endemic areas and guide direct management efforts. Moreover, they help with early detection of CWD, so timely dissemination of information and necessary action can be taken.[67] For example, early detection of CWD in two captive herds and two wild deer in New York in 2005 prompted immediate actions that appear to have successfully mitigated CWD.[152] However, for those regions where self-sustaining CWD epizootics continue to be a challenge, as in the state of Illinois, surveillance efforts have shown that continued intensive management, focused on specific areas infected with CWD, is a powerful management strategy that helps to keep disease prevalence low.[98] There are two types of surveillance for CWD monitoring in free-ranging cervid populations: passive surveillance (which include the testing of road kills, dead, sick or suspect deer for CWD) and active surveillance (which include testing of hunter-harvested deer for CWD in target areas). Although knowledge gaps in the epidemiology of CWD still exist, only continued surveillance will inform CWD management and control strategies.

Science-based policies will help to develop effective management strategies that are relevant to the population monitored. The development of long-term sustainable management strategies is necessary in order to keep low prevalence and to avoid dissemination of CWD. Left unmanaged infection rates will affect the ability of cervidae herds to sustain themselves.[94] CWD regulations to prevent further spread include restriction in translocation of captive cervids and movement of hunter-killed big game carcasses, high-risk tissues or bodily fluids that tend to concentrate high levels of PrP^{SC}. Prion deposition in mineral licks was demonstrated in an enzootic area in Wisconsin, corroborating the participation of mineral licks as risk factors for CWD transmission, environmental reservoirs for CWD prions and as potential sources for cross-species contamination as they attract livestock and non-cervid wildlife species.[101] The bans on baiting and feeding implemented in multiple states in North America and municipalities in Norway are crucial to reduce the congregation of animals and to reduce direct contact rates of susceptible with infected animals that in normal circumstances do not congregate and feed on the precise same small area.[153] This regulatory ban helps to prevent direct transmission of infectious diseases like tuberculosis, brucellosis and CWD.

Conclusions

Chronic wasting disease (CWD) is a highly contagious prion disease that affects captive and free-ranging cervids. The infectious agent is the misfolded prion protein (PRP^{SC}), which is primarily transmitted horizontally via direct contact between animals or indirectly through contact with infective secretions and contaminated fomites. CWD is epizootic in the US and continues to expand

through North America; outbreaks in Europe are on the rise. Our current understanding of the long-term effect of CWD on free-ranging populations is still limited. However, we know the population-limiting impact of CWD, even at low prevalence rates it affects the possibility of a herd to thrive. CWD is a 100% fatal slowly progressive neurodegenerative disease, with long incubation periods wherein sub-clinical-infected animals contribute to the shedding of the pathogenic PrP^{SC}. Given the longevity of infectious proteins in the environment and that the disease is in a free-roaming population, managers are unlikely to completely eliminate the disease, but they can control it. Some management approaches have helped sustain low CWD prevalence and slow the spread of the disease. Genetic tools identify animal movement patterns and population level susceptibility (ie, herd immunity), helping to contain or reduced areas affected by CWD. Yet, gaps in knowledge still exist. An improved understanding of population dynamics, deer behavior that influence CWD transmission among free-ranging cervids and prions in the environment is needed to facilitate CWD management. The effect of protective haplotypes that may be acting as a genetic barrier preventing the spread of CWD, potential therapeutic strategies that will help to protect and manage captive and free-ranging populations, as well as new tools for effective antemortem detection, environmental clean-up and prion protein degradation are all integral components of the future management of CWD. Regardless of the strategy, management of an infectious disease such as CWD is a joint responsibility that involves the government, state and local agencies, farmed cervid producers, hunters and the general public. The key to the success of CWD management in free-ranging deer involves public acceptance and a continual support and commitment to intervention. Only through ongoing scientific research and management based on scientific evidence can CWD be controlled. In the future, if a treatment or cure is identified, our chances to take advantage of those tools will be much better if CWD has been contained and prevalence rates are low.

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