Chronic Wasting Disease of Cervids: Current Knowledge and Future Perspectives

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Abstract

A naturally occurring transmissible spongiform encephalopathy (TSE) of mule deer was first reported in Colorado and Wyoming in 1967 and has since spread to other members of the cervid family in 22 states, 2 Canadian provinces, and the Republic of Korea. Chronic wasting disease (CWD), caused by exposure to an abnormally folded isoform of the cellular prion protein, is characterized by progressive neurological disease in susceptible natural and experimental hosts and is ultimately fatal. CWD is thought to be transmitted horizontally in excreta and through contaminated environments, features common to scrapie of sheep, though rare among TSEs. Evolving detection methods have revealed multiple strains of CWD and with continued development may lead to an effective antemortem test. Managing the spread of CWD, through the development of a vaccine or environmental cleanup strategies, is an active area of interest. As such, CWD represents a unique challenge in the study of prion diseases.

INTRODUCTION

Chronic wasting disease (CWD) is a naturally occurring transmissible spongiform encephalopathy (TSE) affecting members of the cervid species, including white-tailed and mule deer, wapiti, and moose. As with other TSEs, including scrapie of sheep, transmissible mink encephalopathy, bovine spongiform encephalopathy, variant and sporadic Creutzfeldt-Jakob disease (CJD), and kuru, CWD is characterized by central nervous system pathology mediated by an abnormal isoform of the normal cellular prion protein (PrP^{C}). The cellular prion protein, encoded by the *Prnp* gene, is normally composed of multiple α-helices, though through mutation or coercion by an abnormal exogenous or endogenous isoform, it may convert to a tertiary structure that is primarily β-sheet in nature. This structural change renders it resistant to manifold physical conditions, including protease digestion, extreme temperatures, and standard disinfection protocols. It is this resistance to degradation that has led to the designation PrP^{res} for the abnormal prion isoform, also denoted as PrP^{d}, for disease-causing prion protein.

NATURAL HISTORY OF CHRONIC WASTING DISEASE

CWD was first identified in 1967 in a closed herd of captive mule deer in studies conducted jointly through Colorado State University and the University of Wyoming. Initially reported as a syndrome of wasting and progressive neurological disease, CWD was ultimately classified as a TSE in 1980 (1). While at that time CWD was limited to captive mule deer, the disease was later identified in captive and free-ranging wapiti, mule and white-tailed deer, and moose (2–5). Although originally restricted to a loosely defined area of northern Colorado and southern Wyoming, the epidemic has since expanded laterally to Utah, Nebraska, Kansas, and South Dakota, with new epidemic foci identified in southern Wisconsin and northern Illinois, northern West Virginia, Maryland, Pennsylvania and Virginia, southern New Mexico, and far west Texas, as well as the Canadian provinces of Alberta and Saskatchewan (6–13). Smaller foci have been reported in 11 other states and the Republic of Korea (14, 15). Up to half of these foci have arisen in and were limited to farmed cervids, only rarely spilling over to or independently identified in sympatric free-ranging cervids (7, 16) (**Figure 1**).

STRUCTURE OF THE CERVID PRION GENE AND PROTEIN

The cervid prion gene, *Prnp*, like other mammalian prion genes, is a member of the *Prn* gene family, which also includes Doppel (*Prnd*) and Shadoo (*Sprn*) (17, 18). The *Prnp* gene is highly conserved among mammals and in deer spans three exons, with the third exon encoding the open reading frame (19–21). An unexpressed, processed pseudogene ($Prnp^{\psi}$) has also been described in white-tailed and mule deer, though it has no reported relationship to susceptibility or pathogenesis (21, 22).

The normal cervid prion protein (PrP^{C}), as with its gene, is highly conserved among mammals (>90%), with high sequence identity to the cellular prions of reptiles and birds as well (≥30%) (19, 23). The translated protein consists of ~256 amino acids, with signal peptides at both the amino and carboxyl termini, which direct transport to the endoplasmic reticulum, and addition of a glycophosphatidylinositol anchor for cell membrane attachment, respectively (24, 25). The amino terminus, roughly 100 amino acids in length, lacks any definite tertiary structure but does harbor a series of five octapeptide repeats (e.g., GGWGQPHG), which are thought to be involved in binding divalent metals (26). The carboxyl terminus, in contrast, is very highly structured, with three α-helices and two short, antiparallel β-sheets. Typical to other mammalian prion proteins, the carboxyl terminus also has residues to allow for glycosylation and a single disulfide bridge (**Figure 2**).

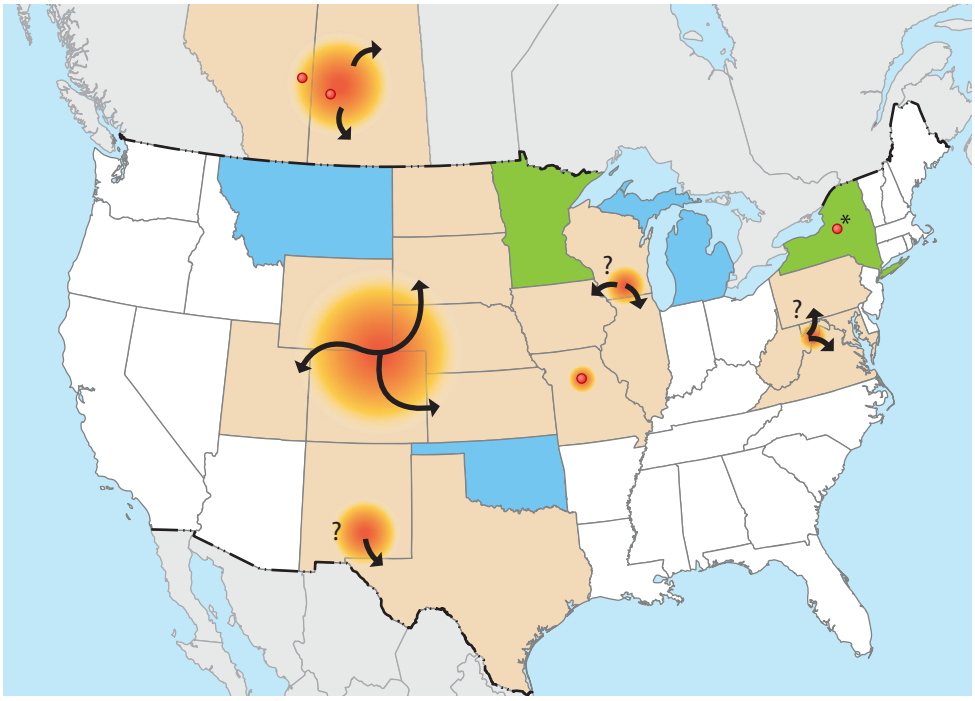

Figure 1

North American distribution of chronic wasting disease (CWD). With an original epidemic focus in northern Colorado and southern Wyoming, CWD has slowly proliferated in captive and free-ranging populations and has now been reported in 22 states and 2 Canadian provinces, as well as the Republic of Korea. States colored tan have ongoing CWD persistence in free-ranging populations, whereas those in blue have limited infection to rare cases in farmed cervids. States in green have reported CWD in wild cervids, though at present indications are that the disease has been eradicated from free-ranging populations. Local foci of infections believed to have originated in farmed cervids are indicated by a red dot, whereas New York State (*asterisk*) represents an unusual circumstance where the disease is thought to have arisen in farmed cervids, spread to wild cervids, and was subsequently eradicated. Question marks indicate foci for which the origin and dispersion patterns, indicated by arrows, are unknown.

The function of the cervid prion protein is unknown, though multiple studies in *Prnp* knockout mice and cattle, and the identification of a group of goats naturally lacking prion protein expression, have shown that the gene is not required for survival. Phenotypically, PrP^{C}-knockout mice may exhibit altered sleep patterns, sensory deficits, and various nervous and immune system pathologies (27–30), whereas both goats with a premature stop codon in the *Prnp* gene and $PrP^{0/0}$ cattle appear clinically and physiologically normal (31, 32).

GENETIC CORRELATES OF INFECTION IN CERVIDS

In early studies of scrapie in mice, it was discovered that variations in the *Sinc* (scrapie incubation period) gene—later identified as the *Prnp* gene—affected the incubation period of infection (33). These findings were eventually applied to investigations in sheep, where several *Prnp* alleles were found to affect susceptibility or resistance to scrapie infection (34–36). Since then, allelic variants have been identified in cattle (37), humans (38, 39), and more recently cervids, as has been

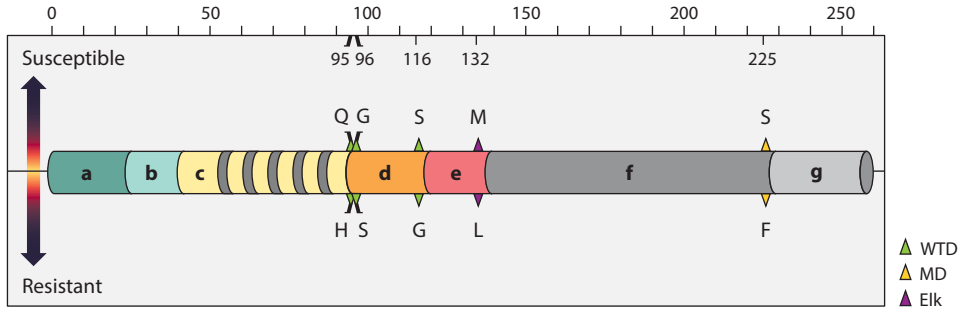

Figure 2

Structure of the cervid prion protein and important allelic variants. The cervid prion protein may be divided into several distinct regions: signal peptides directing transport to the endoplasmic reticulum and addition of a glycophosphatidylinositol anchor (a and g, respectively), areas of charged amino acids (b and d), a series of five octapeptide repeats (c), a region of hydrophobic amino acids where the protein is thought to span the cell membrane (e), and a carboxyl-terminus sequence where organized tertiary folding and posttranslational processing (e.g., glycosylation) are thought to occur (f). Specific amino acids crucial for chronic wasting disease (CWD) resistance or susceptibility are indicated for each species studied to date: In white-tailed deer, 95H, 96S, and 116G have independently been identified to promote resistance to CWD, whereas 132L and 225F are thought to impart resistance in elk and mule deer, respectively. Abbreviations: MD, mule deer; WTD, white-tailed deer.

reviewed recently (40). These variants may influence free-ranging population dynamics and could be relevant to the cervid production industry (see Management, below) (41).

Of the four identified polymorphisms in white-tailed deer (Q95H, G96S, A116G, and Q226K), three have been found to be overrepresented in field cases of CWD: 95Q, 96G, and 116A (40) (**Figure 2**). Interestingly, the alternate alleles are each found at low to very low frequencies in free-ranging white-tailed deer (<~25% of animals genotyped). Although cervids with a 96S allele have been shown to have extremely prolonged incubation periods (130–230% of that of 96GG homozygotic controls), transgenic mouse experiments found the 96S allele actively inhibited infection, with 96S homozygotes strongly resistant to infection (42, 43). It is not surprising, then, that this locus may contribute to selection pressure in CWD-endemic areas (41).

Relatively fewer alleles governing susceptibility in mule deer and elk have been identified. Mule deer with the 225F allele, again found at a very low frequency in the wild, have been reported to have prolonged incubation periods as compared with 225S homozygous mule deer (44). Codon 132 in elk (e.g., M132L), with relatively higher frequency in natural populations, has also been reported to influence susceptibility (45); however, further studies are necessary to verify that 132M homozygotes are indeed more susceptible to infection.

GEOGRAPHICAL DISTRIBUTION OF CHRONIC WASTING DISEASE

The geographical distribution of CWD may be considered as two lineages: (*a*) the slow proliferation of disease among free-ranging cervids, with often stable prevalence and slow rates of diffusion, and (*b*) the dispersion within herds of captive cervids. Epidemic foci among captive herds are typically limited in duration and prevalence; however, in unusual circumstances, prevalence may approach 80–90% of animals and only rarely crosses over into free-ranging populations (1, 16, 46–48).

Among free-ranging cervids, CWD has been identified in 19 American states and 2 Canadian provinces. Initially, the primary endemic zone was limited to northeastern Colorado and

southeastern Wyoming in the United States, though in 1996 a new focus was identified in Saskatchewan, Canada (49). A third endemic focus was later identified in southern Wisconsin in 2002 (50), and eventually separate foci were reported in New Mexico, New York, West Virginia, Missouri, and Minnesota. Very little is known about the epidemiology behind these foci—whether they are extensions of the original endemic focus or secondary foci, or if they represent a distinct, spontaneous development of prion disease in cervids. In any case, the disease has slowly diffused from the endemic zones in each of these locales, apparently following riparian and sylvan corridors (51).

The propagation of CWD in captive herds has followed a slightly different path. Identification in farmed cervids has typically occurred in areas without previous reports of CWD, where it is almost always contained on site. Occasionally, identification of CWD in captive herds precedes or overlaps with the detection of the disease in free-ranging animals, including Saskatchewan (1996–2001), New York State (2005), and Missouri (2010) (7, 8, 48, 52). Often this is an implied spillover of infection from captive to free-ranging animals (53); however, without the appropriate tools to distinguish CWD prion strains or more thoroughly study the focus epidemiologically, this may remain speculative, as it is also plausible that CWD may have moved from free-ranging deer into captive populations in these instances.

HOST RANGE OF CHRONIC WASTING DISEASE

Following its initial report in mule deer, the known host range of CWD has expanded to encompass most of the members of the family Cervidae. Not long after its initial description as a wasting disease of captive mule deer, CWD was also found to affect wapiti, or Rocky Mountain elk, in Colorado in 1981 (2) and was later identified in white-tailed deer in Nebraska and South Dakota in 2001 (54). Long thought to be resistant to CWD, in 2005 a free-ranging moose (*Alces alces*) harvested by a hunter in Jackson County, Colorado, tested positive for the disease (4). Subsequently, CWD was reported in captive moose in Wyoming and again in free-ranging moose in Colorado and Wyoming, and recently in Alberta, Canada (5, 55, 56). The total number of moose reported with CWD has been very low ($n = 6$) relative to the thousands of deer and elk identified over the past three to four decades.

Although not sympatric to deer, elk, or moose in the CWD endemic area, other cervid species have proven to be experimentally susceptible to CWD following experimental challenge. These include Eurasian red deer (*Cervus elaphus*) (57) and fallow deer (*Dama dama*) (58), Asian muntjac (*Muntiacus reevesi*) (59), and arctic/subarctic reindeer or caribou (*Rangifer tarandus*) (60). Each of these species has both captive and free-ranging counterparts across the globe and, because of their susceptibility, should be considered in routine CWD/TSE surveillance.

PATHOGENESIS IN THE NATURAL HOST

Much of what has been reported on the pathogenesis of CWD has been predicted from studies on sheep scrapie; indeed, the two TSEs show remarkable similarity in their modes of transmission (via direct contact or contaminated environmental fomites), incubation period (18 months–5 years), and pathology (54, 61), and as such, an extrapolation of pathogenesis of CWD from scrapie studies and vice-versa seems justified, albeit not absolute. The infectious CWD prion is thought to initially cross the alimentary epithelium after ingestion of contaminated material and progresses relatively quickly to lymphoid centers associated with the alimentary tract, including tonsil and retropharyngeal lymph nodes (RLNs) (62). It is thought that the sympathetic fibers associated with the germinal centers of these lymphoid tissues direct prion proliferation to the central nervous

system, though it is worth noting that in a sizable percentage of elk (10–15%), no evidence for lymphoid involvement can be found using conventional methods (54). From these ascending fibers of the autonomic nervous system, the infectious prions and PrP^d are typically found along with spongiform degeneration of the dorsal motor nucleus of the vagus nerve in the obex region of the medulla oblongata. Progressive and more widespread prion deposition throughout the central nervous system is thought to occur very close to the onset of clinical disease, based on studies that systematically scored central nervous system tissues (63, 64).

A key component of pathogenesis that requires further investigation is the accumulation of PrP^d in peripheral tissues, e.g., nasal mucosa, tonsil, salivary glands, taste buds, kidneys, urinary, bladder, intestine, and rectoanal mucosa–associated lymphoid tissue, and its association with the onset of shedding in excreta. Peripheral accumulation has been thought to occur only after central nervous system replication; however, recent findings have shown that shedding in excreta may begin concurrent with peripheral lymphoid accumulation (N.J. Haley, unpublished data). Although examination of peripheral tissues from end-stage animals has demonstrated amplifiable CWD prions in peripheral tissues, a more thorough serial analysis of peripheral tissues over the course of infection would shed greater light on the timing of CWD appearance in the periphery.

CLINICAL AND PATHOLOGICAL SIGNS

Following a variable incubation period (dependent on, for example, exposure dose and genotype), experimentally infected cervids may exhibit clinical periods over a few weeks to a few months (65–67). Earliest symptoms of clinical disease include behavioral and subtle locomotive changes. Subsequent signs include progressive weight loss, bruxism, altered posture and head placement, head tremors, and ataxia. Paradoxical polydypsia and polyphagia in the face of weight and muscle mass loss are hallmarks of the symptomatic phase of CWD. Interestingly, symptoms of sialorrhea and polydypsia/polyuria likely contribute to late-stage shedding of infectious prions (65, 68). Although the progress of clinical disease is less documentable in wild cervids, CWD-positive animals are more likely to succumb to vehicular and predator-related fatalities, likely owing to subclinical neurological disease (69, 70).

It is very difficult to diagnose CWD on gross necropsy, as many of the signs of the disease are nonspecific (54). Despite its nomenclature, CWD-positive cervids may often have average body condition, as weight loss typically occurs in later stages of disease, and wild cervids may succumb to earlier fatal factors (49, 54, 69–71). Affected cervids may be considered in poor thrift with roughened hair coats; additionally, esophageal dilatation, regurgitation, and evidence of aspiration pneumonia may be present (54).

On microscopic examination, neuronal degeneration and astrocytosis are prominent findings bilaterally in the gray matter of the diencephalon, olfactory cortex, and medulla (54, 72). The dorsal motor nucleus of the vagus, acting as the proposed conduit between peripheral exposure and central disease, is an area of the central nervous system commonly demonstrating spongiform degeneration early in the course of infection. Amyloid plaques, occasionally florid in nature (i.e., among vacuolar degeneration of the neuropil), are common, in contrast to the granular plaques common to bovine spongiform encephalopathy (BSE). Immunohistochemistry (IHC) demonstrates widespread, and especially perineuronal and perivascular, PrP^d reactivity. Lymphoid tissues, e.g., lymph nodes and spleen, demonstrate PrP^d deposition in germinal centers and follicles, with increased accumulation and dissemination over the course of disease. PrP^d may also be detectable in neuroendocrine tissues throughout the body, including pancreatic islet and adrenal medullary cells and enteric ganglia and nerve plexuses (73).

CHRONIC WASTING DISEASE IN EXPERIMENTAL, NONTRANSGENIC HOSTS

CWD has been experimentally transmitted through various routes (e.g., intracerebral inoculation) to various species, including rodents (voles, *Myodes glareolus*, and hamsters, family Cricetidae) (74–76), carnivores (ferrets, *Mustela putorius*, and cats, *Felis catus*) (77–79), other ruminants (cattle, *Bos primigenius taurus*) (80, 81), and nonhuman primates (squirrel monkeys, *Saimiri sciureus*) (82, 83). These species have served as both model species for the study of CWD pathogenesis (e.g., ferrets and hamsters) and experimental species for the study of transmission barriers relevant to human and livestock health, or to identify potential natural reservoir species (e.g., voles). In many cases, CWD isolates were subpassaged and adapted to allow correlation to known species-specific TSEs (e.g., BSE, transmissible mink encephalopathy, and feline spongiform encephalopathy), as well as to analyze prion protein alterations associated with trans-species infection and replication.

In rodents, several studies have evaluated the susceptibility of common laboratory species (e.g., hamsters) or wild species (e.g., voles) to CWD. Relying on intracerebral inoculation, these studies have demonstrated relatively short incubation periods (6–12 months), with a variety of species-typical clinical and strain-specific pathological findings. Both hamsters and voles exhibited clinical patterns of progressive neurological dysfunction, including head bobbing and ataxia, though the observed duration of signs is reported to be quite short (75). Central nervous system pathology characteristic of TSEs, specifically moderate spongiosis, astrocytosis, and neuronal loss, were observed in rodents, whereas biochemical analysis showed subtle changes between cervid and rodent-passaged PrP^d (75, 76).

Ferrets have been studied as an experimental model for CWD pathogenesis in the past decade, though their utility has waned with the introduction of transgenic murine model systems (84). On primary passage, incubation periods in ferrets are typically long (15–20 months), with successful transmission observed only in intracerebrally inoculated animals (79, 85). Clinical disease, distinct from those seen in mink with TME, was observed over several weeks, with animals demonstrating isolation, polyphagia, and somnolescence, progressing to torticollis and ataxia. Subsequent passages of ferret CWD led to more facile oral transmission and incubation times ranging from 4.5 to 9 months, correlating to various CWD isolates (see below, Strains of CWD) (77, 85, 86). Microscopic examination of central nervous system tissues found spongiosis in the basal ganglia, thalamus, optic chiasm, midbrain, and pons. PrP^d aggregates were small, coarse, and neuronally associated. Ferret-adapted CWD showed similar lesions as primary passaged material, though they were more extensive in nature. In contrast to what has been reported in other experimental hosts, western blot analysis demonstrated ferret PrP^d glycoform patterns similar to those seen in parent deer isolates (86).

In 1988, a novel TSE among domestic and wild felids was identified and correlated to exposure to BSE-contaminated feedstuff: feline spongiform encephalopathy (87–89). Accordingly, studies evaluating the susceptibility of domestic cats to CWD were undertaken. Through intracerebral inoculation, primary passage of CWD into cats was found to have very long incubation periods (45–47 months) and low attack rates (40%) (78). As was observed in ferrets, oral transmission was unsuccessful. On secondary passage, however, incubation periods were drastically shortened (23–27 months), with complete attack rates and the renewed potential for oral transmission. Affected cats showed gait abnormalities, weight loss and anorexia, and polydypsia, along with aggression and hyperreactivity. Pathological lesions were observed primarily in the obex and caudal brainstem, including the dorsal motor nucleus of the vagus, with more extensive and heterogeneous lesions seen on secondary passage. PrP^d aggregates were finely granular and perineural to

intraneuronal, similar to that reported in ferrets, though distinct from neuropathological profiles observed in either CWD or classic feline spongiform encephalopathy (90). Glycoform patterns showed a lower molecular weight and altered immunoreactivity compared with parent CWD strains (78). Work is currently ongoing to identify whether sympatric felid species (e.g., North American cougar, *Puma concolor*) may be susceptible to CDW infection (M. Miller, personal communication).

In an effort to evaluate the susceptibility of domestic cattle to CWD, several studies evaluating various isolates of CWD (e.g., mule deer, white-tailed deer, and elk sources) through multiple passages have been reported (80, 81, 91). Incubation periods in intracerebrally inoculated cattle have been variable and isolate dependent, ranging from 18 to 63 months and demonstrating variable attack rates. To date, no evidence of oral transmission of CWD to cattle has been reported (58, 80, 81). Clinical signs in affected cattle range from subtle signs to signs similar to those of BSE: circling, weight loss, bruxism, and general depression. Central nervous system lesions were infrequent, though multifocal PrP^d staining in particulate or granular deposition patterns proximal to astrocytes, with occasional small aggregates or plaques, was observed. These findings are distinct from the classic spongiform lesions and diffuse PrP^d distribution in linear and granular patterns in the neuropil of cattle affected with BSE (91). The PrP^d molecular weight profiles seen in bovine CWD were reportedly lower than those observed with both parent CWD strains and BSE, with very little apparent difference in glycoform distribution.

Given the definitive link between BSE and variant CJD, several studies assessing the susceptibility of primates to CWD have also been undertaken. Although evidence from transgenic, humanized PrP mice showed a likely species barrier (92), thorough studies by several groups have shown that some primate species may in fact be susceptible to CWD infection. Transmission studies in rhesus macaques have to date been negative; however, squirrel monkeys have been found to be susceptible to CWD following both oral and intracerebral inoculation (82, 93). Incubation periods were generally long (31–53 months), with low to moderate attack rates (3/15 animals and 11/13 animals following oral and intracerebral inoculation, respectively). Lesions were observed throughout the central nervous system, including spongiosis and variably sized plaques. Interestingly, subpassage of squirrel monkey tissues into tgDeerPrP mice failed to produce infection, possibly indicating a loss of host range (82). Despite the results in New World monkeys, to date no reports of an increased risk of CJD have been reported in individuals consuming venison, with both retrospective and prospective studies reported in the literature (94–96).

TRANSMISSION OF CHRONIC WASTING DISEASE AND ENVIRONMENTAL PERSISTENCE

CWD's efficient horizontal transmission has been an active area of research in recent years, and both environmental contamination (and persistence) and excreta (e.g., saliva, urine, and feces) are thought to play a pivotal role in the rapid proliferation of CWD across North America over the past five decades. Initial studies by Miller and colleagues (66) clearly demonstrated that naïve mule deer exposed to infected cohorts would eventually develop and succumb to CWD. Moreover, the physical presence of an infected animal was not required for transmission; animals housed in pens that previously held infected animals were also at risk, implying fomites contaminated through excreta or decomposing carcasses also contributed to horizontal transmission (66).

Building from this study, Mathiason et al. (65, 67) focused on specific forms of excreta by exposing naïve cervids to saliva or urine and feces from preclinical or clinically ill, CWD-positive deer. In these studies, saliva showed the clearest potential for contributing to horizontal

transmission: Of six deer exposed to saliva by the oral route, five became infected as early as 12 months postexposure, as evidenced by antemortem tonsil biopsy IHC. Clinical, IHC, or western blot evidence of CWD infection has not been reported in several deer inoculated with urine and/or feces following 18+ months of incubation, though later bioassay and in vitro amplification studies provided evidence that many of these deer were subclinically infected at the time of necropsy (97). Further studies in transgenic, cervidized tg[CerPrP] mice found that urine and feces (as well as saliva) were overtly infectious in this experimental mouse model (68, 98). Oftentimes, in vitro amplification assays have supported these in vivo findings—with PrP^d demonstrated by seeded amplification in each form of excreta (68, 99, 100).

Transgenic, cervidized mice have also been integral in narrowing down the onset of shedding in bodily fluids. Recent research systematically evaluating saliva, urine, and feces has demonstrated that infectivity in saliva and feces may initially appear as early as 9–12 months postexposure, long before the onset of clinical signs (N.J. Haley, unpublished data; 98). Infectivity in urine, however, was observed at very low levels in samples collected very late in disease. Calculations of cumulative infectivity doses in each form of excreta approached that found in the central nervous system, emphasizing their role in the persistence of infection in cervid species (N.J. Haley, unpublished data; 98, 101).

The spread of infectious prions through excreta and decomposing carcasses is likely only part of the transmission puzzle, however. Unlike many other infectious diseases, the prion agent is remarkably stable in the environment, persisting for many years at sites housing TSE-affected animals (66, 102). Although this persistence likely is partly due to the protein's hardy biochemical properties, much research in recent years has gone into investigating its ready binding to soil (103–106). Cervids and other ruminants, through grazing behaviors, have very close contact with the soil, so it is perhaps unsurprising that research has been directed at identifying which particular soil components may be involved in prion persistence and the kinetics under which prion/soil binding occurs.

Through a solution-depletion approach, by which the amount of PrP^d in solution is measured before and after addition of soil, researchers have found that infectious prions may bind rapidly and vigorously to various minerals and soil compositions, especially montmorillonite clay and quartz (106). Interestingly, these soil types have been found to very closely correlate with prevalence of CWD in endemic areas (107, 108). The flexible amino-terminus, with a higher proportion of positively charged amino acids, has been found to be crucial to soil binding; proteinase K digestion, which cleaves PrP^d within this flexible N terminus, has been used to dissociate bound prions from soil for analysis (109). Once bound to soil, the prion protein has very little mobility, potentially keeping it very close to the soil surface and perhaps even enhancing ruminant contact (110).

Soil binding may provide one other advantage to the infectious prion protein: Some studies have found that soil-bound prions may in fact be more infectious than unbound prions in mouse models, reducing incubation periods and increasing attack rates (103, 104). Other researchers have found that soil-bound prions may instead reduce infectivity in both in vivo and in vitro models of infectivity (111). Infectivity may ultimately relate to the type of soil bound to the infectious prion protein, though at the very least the prion-soil relationship likely allows infectivity to persist for decades (66, 102, 111).

EVIDENCE FOR CHRONIC WASTING DISEASE STRAINS

Although many TSEs tend to be relatively species specific, various different strains or isotypes have been identified within each clade of disease, e.g., scrapie (263K, ME7, RML, classical and atypical

scrapie), BSE (atypical BSE - BASE), and CJD (kuru, CJD, variant CJD, Gerstmann-Straussler-Scheinker syndrome). CWD is not unique among TSEs, in that there have been multiple reports outlining evidence for strains of CWD (76, 85, 112). These strains may be partially species specific (i.e., relatively more or less common in deer or elk), or potential quasispecies or multiple conformers, replicating independently within an individual animal.

The criteria for distinguishing CWD strains are primarily based on several metrics from passage of isolates in susceptible hosts: (*a*) average incubation periods, (*b*) range of clinical signs, (*c*) the distribution of central nervous system pathology, and (*d*) PrP^{d} biochemical characteristics. Based on bioassay studies, including passage of CWD into ferrets, hamsters, and cervidized mice, at least two putative strains of CWD have been proposed (e.g., CWD-CSU and CWD-WI; $SghaCWD^{md\text{-}f}$ and $SghaCWD^{md\text{-}s}$; CWD1 and CWD2) (76, 85, 112) (**Table 1**). Although each of these isolated strains has not been evaluated in cross-species experiments to verify a consistent phenotypic pattern, one strain typically produces a much shorter incubation period (e.g., approximately five months in ferrets, approximately three months in hamsters, and approximately seven months in transgenic mice) and a wide distribution of PrP^{d} deposition, whereas the second has a somewhat longer incubation period and a more limited PrP^{d} lesion profile. Attack rates, clinical signs, and disease progression may also vary between CWD strains, although the biochemical properties (e.g., glycosylation pattern and guanidine hydrochloride denaturation profiles) are often indistinguishable between the two isolates.

CONVENTIONAL AND EXPERIMENTAL DETECTION OF CHRONIC WASTING DISEASE PRIONS

Since the original reports of CWD in 1967 and its initial classification as a TSE in 1980, the detection of infection has improved significantly from a purely histopathological diagnosis to seeded amplification assays, such as protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) (1, 100, 113). Despite the lack of a nucleic acid sequence—the basis of assays such as polymerase chain reaction—these in vitro prion amplification methods are quickly taking the place of in vivo infectivity assays. Prion disease detection has also evolved from strictly postmortem sampling to increased interest (and success) in antemortem diagnosis, offering the potential for testing prior to animal movement and surveillance of susceptible threatened or endangered species (114–117).

The early detection of CWD infection was limited to microscopic evaluation for the classic TSE pathological signature: spongiform degeneration, astrocytosis, and florid plaques that may

Table 1 Summary of evidence for strains in experimental cross-species transmission of chronic wasting disease

Experimental species	Strain identification	Incubation period (range)	Central nervous system pathology	Reference
Ferrets (*Mustela putorius*)	CWD-WI	8.4–15.9 months	Substantial PrP^{d} deposition	85
	CWD-CSU	3.5–6.6 months	Comparably limited PrP^{d} deposition	
Syrian hamsters (*Mesocricetus auratus*)	$SghaCWD^{md\text{-}f}$	85–89 days	Widespread PrP^{d} deposition and gliosis	76
	$SghaCWD^{md\text{-}s}$	485–544 days	Limited PrP^{d} deposition and gliosis	
Tg[CerPrP] mice	CWD1	225+/−18 days	Symmetrical PrP^{d} deposition and gliosis	112
	CWD2	301+/−35 days	Asymmetrical PrP^{d} deposition and gliosis	

be more visible following silver or amyloid binding molecule (e.g., Congo red or thioflavin T) staining (54, 72). The obex, a region of the medulla oblongata proximal to the transition of the fourth ventricle into the central spinal canal and harboring the dorsal motor nucleus of the vagus, as noted above, was identified as one of the earliest sites of central nervous system pathology. Immunohistochemical techniques, and later western blotting and ELISA—first reported more than a decade after its initial classification as a TSE—advanced both clinical and experimental studies on CWD (118–120). The ability to identify CWD-positive animals by immunoassay permitted sensitive and specific diagnosis, rapid surveillance ability, and enhanced pathogenesis studies. Evaluation of peripheral lymph nodes allowed a greater understanding of the involvement of the lymphoid system in the migration of CWD from the gastrointestinal tract to the central nervous system and proved to allow earlier and more sensitive detection of CWD infection in deer through RLN analysis (63). As noted above, RLN evaluation alone may miss up to 10–15% of elk infected with CWD, implying variable pathogenesis among cervid species. As a result, IHC of obex sections may still be considered the gold standard for CWD diagnosis in cervids (54).

Although immunoassays like IHC and ELISA are quite capable of detecting CWD infection with good sensitivity and very high specificity (97, 121), these early in vitro assays were unable to reveal whether or not a given sample was infectious and were not sensitive enough for the identification of CWD prions in body fluids and subclinically infected animals (68, 97). Bioassay, either in the natural host or in transgenic mice developed to express the cervid prion protein, has served as both the standard for prion infectivity and identification of infected tissues and infectious body fluids (84, 122, 123). As mentioned above, transgenic mouse models have been integral to the identification of infectious prions in the central nervous system and lymphoid tissues, saliva, urine, and feces. Following titration of infected brain pools in these mice, it is also possible to estimate the infectious doses present in these samples, providing estimates helpful for disease modeling and mitigation of transmission (98, 101).

Whereas bioassay may be the standard for detecting infectivity, ethical dilemmas as well as the costs of developing and maintaining transgenic models make in vitro amplification and detection systems more enticing. Extrapolating from in vivo work, in vitro assays initially focused on whole-brain homogenates for modeling prion amplification and infectivity (124). By using mouse brain as a substrate for PrP^C conversion, unknown seed or spike samples could be incubated and either shaken or sonicated to disrupt growing amyloid fibrils (124). The disrupted fibrillar prion protein could then go on to form additional fibrils, resulting in an amplification process that enhanced detection sensitivity several orders of magnitude greater than that of conventional western blotting. This PMCA process could be performed over successive rounds with fresh brain homogenate substrate over the course of 7–10 days, with ultimate evaluation by western blotting (125, 126).

To determine the minimal requirements for prion conversion in vitro, other researchers sought to take advantage of recombinant prion protein, in lieu of whole-brain homogenates, as a substrate for prion amplification. The continued pursuance of recombinant prion substrates led to the development of RT-QuIC (127). It is thought that, while the prion seed coerces the recombinant prion isoform into the abnormal β-sheet structure, thioflavin T added to the reaction is incorporated into the growing amyloid. Once bound, thioflavin T exhibits an altered spectrofluorimetric emission pattern, with increases in fluorescence monitored over time—usually 24–60 h (128–130). This approach has very much in common with real-time polymerase chain reaction, with a similar read out, and has allowed the detection of femtogram levels of infectious prions in tissues and body fluids, similar to the lower detection limit of bioassay (129). Based on this sensitivity, amplification assays such as PMCA and RT-QuIC may in many instances replace in vivo assay systems for the detection of ultra-low concentrations of PrP^d. Amplification-specific

reagents, equipment, and expertise will for a time limit the widespread diagnostic application of these methodologies, however.

MANAGEMENT

As a result of differing management objectives, control of the spread of CWD necessarily must be tailored to either of the two populations affected: farmed and free-ranging cervids. The farmed cervid industry is very much like that of any agricultural production industry, and disease management and mitigation have benefited from the common practice of individual animal identification (for example, with cervid and bovine tuberculosis testing) and an annual census, combined with a herd certification approach. Postmortem CWD testing of all animals harvested on the farm or sent to slaughter is common practice. Upon identification of a positive case, herds are placed under quarantine prior to depopulation with or without indemnity; animals are then traced back to identify herd origin and tested for CWD. This level of surveillance far overshadows that available in field situations and is likely to have contributed to the timely identification of positive herds within the cervid industry. It may, however, lead to the false assumption that, because CWD may have been identified in farmed cervids prior to free-ranging cases, an epidemic focus originated on a farm.

The cervid farming industry would surely benefit from other mechanisms of disease control employed in agricultural production, including antemortem testing and enhanced biosecurity. The development of a simpler antemortem test for use prior to animal movement between farms or prior to the sale and distribution of live cervid products, including reproductive samples and antler velvet, would give producers and oversight agencies confidence in the movement of animals and animal products. Breeding for resistant genotypes, as has been increasingly successful in the management of scrapie, may also prove beneficial. Biosecurity measures commonplace in other production animal facilities, including double 8′ fencing, animal quarantine prior to introduction, and equipment cleaning, would be valuable additions to prevention and management strategies.

Management of CWD in free-ranging populations has understandably proven to be much more problematic. Although population reduction is often effective in reducing diseases spread by direct contact, when employed for CWD management it is often unsuccessful, perhaps owing to the magnitude and geographical breadth of the infected populations and underlying environmental contamination (131, 132). In two instances, herd reduction has been putatively effective in CWD control: central New York State and southeastern Minnesota (133, 134). Other attempts in CWD endemic areas, including Colorado, West Virginia, and Wisconsin, have not had any apparent effect on prevalence rates (132, 135, 136). Nonspecific culling, as with government-sponsored sharpshooters, has been reported to be more successful than publically mediated herd reduction (132). The aggregation of wildlife via feeding and baiting practices is sure to increase animal-animal contact and in many areas has been prohibited to lessen the opportunity for CWD spread (137). Control of CWD in the free-ranging cervid population thus represents a unique challenge with unknown consequences among prion disease.

FUTURE PERSPECTIVES

Despite the long-standing recognition of CWD and the wealth of progress made in the understanding of pathogenesis, transmission, and detection, several areas of research still require attention: (*a*) the development of a true live-animal test with sensitivity greater than or equal to that of postmortem IHC or ELISA, (*b*) the ability to distinguish strains rapidly in vitro, (*c*) the construction of a successful vaccine target and strategy, and (*d*) the implementation of an effective

environmental clean-up protocol. Advances in each of these areas would go great lengths in curbing the spread of CWD across North America.

Live-Animal Test

Many approaches have been described for preliminary detection of CWD infection antemortem, including IHC of either tonsilar or rectal lymphoid tissues; however, each of these has been found to have somewhat reduced sensitivity when compared with terminal obex or RLN IHC (47, 117, 138). Blood could be the optimal antemortem collection sample; however, several hurdles have overshadowed the detection of CWD prions in blood: (*a*) Little is yet known about the kinetics of prionemia in CWD-affected deer, which affects when, during the course of infection, blood sampling may be ideal or useful, and (*b*) while blood has proven to be a somewhat difficult sample type in the various amplification assays, recent successful results have been reported using RT-QuIC. The assay has also shown promise for use with saliva and urine samples from CWD-infected deer. More work will be needed to apply these approaches in larger-scale, blinded studies with samples from cervids at various stages of infection (100, 139).

Feces, easily found throughout bedding and grazing areas frequented by cervid species, would be ideal for surveillance in areas where CWD incursion is a concern. This approach would also offer the benefit of precluding the need for physical contact with animals through sedation or restraint; indeed, fecal sampling could be done even during the seasonal absence of cervids in traditional grazing areas. A small number of studies have used fecal samples from elk with some success (99), although more work on this approach is certainly needed. Nasal brush samples have also shown limited success in the screening of free-ranging elk for CWD infection by RT-QuIC (N.J. Haley, unpublished data; 140). Brush samples have proven relatively simple to collect and process in both elk and farmed cervids, though, as with other antemortem samples, very little is known about the timing of PrP^d appearance in nasal epithelium, and diagnostic sensitivity and specificity are unknown without a large-scale, blinded study.

Strain Typing

With the further distribution of CWD across North America, and the increased frequency with which the disease is identified in captive herds, it is becoming increasingly important to develop a technique to strain-type CWD isolates. The two reported strains of CWD exhibit no overt biochemical differences, making conventional in vitro strain discrimination (e.g., molecular weight differences and glycosylation profiling) difficult, whereas the use of mouse bioassay techniques is both cost and time prohibitive (112). Several methods have been described recently for the in vitro discrimination of prion strains, though they have yet to be applied to CWD.

The first technique uses luminescent conjugated polymers, compounds that, when bound to PrP^d, emit a fluorescence spectrum that is dependent on prion conformation (141). This approach may successfully differentiate various families of TSEs, including BSE, scrapie, and CWD, but so far has not been used to evaluate different strains of CWD. In theory, these polymers could also be incorporated into the RT-QuIC assay, where their discriminatory capabilities may prove useful in differentiating CWD strains in real time. A second technique attempts to discriminate various TSE isolates by Fourier transform-infrared spectroscopy (FT-IR), which provides a PrP^d fingerprint based on the strength of hydrogen bonds and β-strand packing (142, 143). PrP^d could be prepared directly from CWD isolates or, if at very low levels in tissues, amplified in serial PMCA prior to FT-IR analysis. This approach has recently shown promise in discriminating natural and artificial strains of scrapie in hamsters (144) and could be applied to various isolates of CWD from captive and free-ranging cervids around the country.

Vaccination

The development of an effective CWD vaccination strategy has been hindered by problems common to TSE vaccination—overcoming the natural barrier to antibody development against self-antigen and subsequently limiting the resulting immune response to the abnormal prion isoform. Despite these hurdles, several elegant approaches are in various stages of development for a vaccine against CWD infection. A recent vaccine trial used recombinantly produced segments of the cervid prion protein that had previously shown promise in a mouse model of scrapie (145). Following a prime-boost approach, vaccinated and control deer were naturally challenged via a CWD-contaminated environment. Although deer were found to successfully mount an immune response against the challenge peptides, both vaccinated and control deer eventually succumbed to infection, and the approach was considered unsuccessful.

A second and perhaps more promising approach, using an attenuated *Salmonella* vector expressing the cervid prion protein to generate a primary mucosal immune response, is currently under investigation (146). After oral vaccination and establishment of a detectable immune response, deer were boosted with an inoculation of polymerized, recombinant PrP at regional lymphoid sites (e.g., tonsil and rectal mucosa). Following oral challenge with a CWD-positive brain homogenate, vaccine and control deer were monitored by peripheral biopsy to assess CWD status. Interestingly, a greater proportion of vaccinated deer were reported to be asymptomatic (although the majority were biopsy positive) for CWD 24 months post-exposure, and the mean survival period for the vaccinated deer was significantly longer compared with the sham-vaccinated controls (145). Ultimately, all but one vaccinated and all control deer succumbed to infection. Thus, while intriguing and perhaps promising, more work is needed to explore this unconventional approach for the control of CWD and other prion diseases.

Environmental Clean-Up

The contamination of public wildlife areas, via excreta or decomposing carcasses, and the long-term quarantine of private cervid facilities where CWD has been found make the development and implementation of an effective clean-up regime imperative in both managing the spread of CWD and offering an alternative to farm depopulation and site condemnation. A concise review of the biotic and abiotic approaches to prion inactivation has been published recently, which summarizes nicely several techniques used to remove prions from contaminated environments, including microorganismal composting and protease treatment (106, 147, 148). More work in modeling and assessing the persistence of excreted CWD prions in the environment is needed to assess the risks and practical means for decontamination.

CONCLUSIONS

Much has been learned about CWD since its initial discovery nearly 50 years ago. Pathogenesis studies in both natural and experimental hosts have helped us to understand the centripetal trafficking of prions from the periphery to the central nervous system and the centrifugal dissemination to peripheral tissues, where it is ultimately shed in excreta. These studies have also allowed the recognition that multiple strains of CWD likely exist in nature, whereas epidemiological investigations and studies in similar prion diseases have led to the understanding that certain cervid genotypes may be more or less resistant to CWD infection. Additional work on the development of amplification-based detection assays has expanded our understanding of CWD pathogenesis and transmission, while offering the hope of a live animal test and the added potential of identifying prion strains in vitro. Much work remains to be done, however, with both

effective vaccine and environmental decontamination strategies going a long way toward controlling the spread of CWD across North America.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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