Large animal models for chronic wasting disease

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Abstract

Chronic wasting disease (CWD) is a fatal neurodegenerative prion disease of cervid species including deer, elk, moose and reindeer. The disease has shown both geographic and species expansion since its discovery in the late 1960's and is now recognized in captive and free-ranging cervid populations in North America, Asia and Europe. The facile transmission of CWD is unique among prion diseases and has resulted in growing concern for cervid populations and human public health. The development of native cervid host models with longitudinal monitoring has revealed new insights about CWD pathogenesis and transmission dynamics. More than 20 years of experimental studies conducted in these models, using biologically relevant routes of infection, have led to better understanding of many aspect of CWD infections. This review addresses some of these insights, including: (i) the temporal intra-host trafficking of CWD prions in tissues and bodily fluids, (ii) the presence of infectivity shed in bodily excretions that may help explain the facile transmission of CWD, (iii) mother-to-offspring CWD transmission, (iv) the influence of some *Prnp* polymorphisms on CWD susceptibility, and (vi) continued development of vaccine strategies to mitigate CWD.

Keywords Chronic wasting disease · CWD · Native host model

Introduction

The progressive geographical and host range expansion of CWD across North America, now into Asia and Europe, continues to elicit cervid and public health concerns. CWD poses a clear threat to cervid populations (Edmunds et al. 2016; DeVivo et al. 2017), and the zoonotic potential of CWD remains uncertain (Geist et al. 2017; Barria et al. 2011; Marsh et al. 2005; Comoy et al. 2015; Davenport et al. 2015; Ricci et al. 2017; Wang et al. 2021). The US Fish and Wildlife Service estimates that in 2016 alone, 9 million Americans (1 in 36) pursued big game such as deer and elk with ~\$26 billion spent on hunting (Service USFaW 2016). The human health risks from CWD are a growing concern in that it is estimated that between 7,000 and 15,000 CWD-infected cervids are consumed by humans annually (increasing ~20%/year) (Service USFaW 2016). To date, exposure to CWD has not been shown to result in human

disease. However, given the long incubation periods of related human prion diseases (Prusiner 1982) and the lack of robust surveillance data for either human or animal populations, definitive conclusions are not possible.

CWD discovery and spread over 40 years

Chronic wasting disease (CWD) is a fatal neurodegenerative disease of cervid species (deer, elk, moose and reindeer) that has been identified in captive and free-ranging populations in North America, Korea and Scandinavia (Otero et al. 2021). CWD belongs to the larger family of protein misfolding disorders and is classified as a transmissible spongiform encephalopathy (TSE) or prion disease. The disease was first described in a population of mule deer that were not thriving in captivity (Williams and Young 1980). Originally, it was thought that the stress of captivity and nutritional deficits were the cause of their clinical presentation, which included lethargy and wasting. Post-mortem microscopic examination of brain tissue revealed the presence of lesions and vacuole formation similar to that reported in the brains of sheep with scrapie. These findings led to the diagnosis of a neurodegenerative disease, and the subsequent eponym naming of the condition as “chronic wasting disease.”

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For over a decade after CWD's initial description, the focus was placed on identifying the disease through histological evaluation of post-mortem tissues of infected animals. These studies laid the important groundwork for identifying infected animals and disease progression. Field observations and studies revealed that CWD can be transmitted by direct animal-to-animal contact and by indirect contact with environments that previously housed CWD-infected cervids (Miller et al. 1998, 2004). The authors of these studies postulated that the infectious agent responsible for CWD may be present in bodily secretions, and that the agent was shed in sufficient quantity to initiate infection and disease progression among the herd. These studies also began to allude to the incredible resilience of the infectious prion in the environment, potentially contributing to its transmission in nature. The development and use of a novel CWD specific immunohistochemistry (IHC) assay (Spraker et al. 1997; O'Rourke et al. 1998) revealed CWD is efficiently transmitted within herds. Epidemiological evidence from captive animals suggests that horizontal transmission may occur at levels exceeding that observed in related prion diseases (Miller et al. 1998; Williams and Young 1992). These findings were surprisingly prescient, as CWD has recently been reported in captive herds with prevalence rates up to 80–90% (Rivera et al. 2019), and in free-ranging cervid populations with prevalence as high as 30–50% (Rivera et al. 2019). The consequence of this expansive disease burden is now resulting in cervid population decline (Edmunds et al. 2016; DeVivo et al. 2017).

Evidence supporting expanded geographic and cervid species distribution of CWD has been accumulating. Conventional prion detection methods (IHC, Western blot analysis, and ELISA) have been employed in lymphoid and brain tissue to probe for the presence of the partially proteinase resistant aberrant prion protein (PrP^{res}), a biomarker of prion disease (CWD; PrP^{CWD}). Patterns of PrP^{CWD} accumulation in cranial lymphoid tissues of preclinical cases provided the basis for an antemortem CWD test (Wild et al. 2002) and improved methods for large-scale CWD surveillance (Hibler et al. 2003). Prior to 2000, CWD was known to be present in 5 US States and 1 Canadian Province (Fig. 1). As of the writing of this review, CWD has been detected in 26 US States, 4 Canadian Provinces, South Korea, Norway, Finland and Sweden (Fig. 1) (Rivera et al. 2019; America USGSCmN 2021). Although the dramatic recording of CWD expansion may be in part due to greater surveillance and more reliable detection methods, it is apparent that the disease is spreading among cervid populations in North America and globally. Natural infections have been reported in a variety of cervid species, including mule deer (*Odocoileus hemionus californicus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), moose (*Alces alces*),

red deer (*Cervus elaphus elaphus*) and reindeer (*Rangifer tarandus tarandus*). The well-described facile transmission of CWD led to further query as to how this disease is maintained within cervid populations.

CWD infection progression from exposure, intra-host dissemination, to shedding

Development of native host experimental model systems was critical to characterize the temporal distribution of CWD burden in tissues and secretions of infected cervids to determine their impact on CWD pathogenesis and transmission dynamics. Other non-familial TSEs, such as kuru, transmissible mink encephalopathy and bovine spongiform encephalopathy, have been shown to be transmitted via ingestion of infected tissues (Cervenakova et al. 1998; Marsh and Bessen 1993; Wells et al. 1998). Oral exposure is thus a plausible route of transmission of CWD prions for cervids in nature. In fact, oral inoculation has resulted in productive infections in moose (Kreeger et al. 2006), elk (Hamir et al. 2006; Plummer et al. 2017), white-tailed and mule deer (Plummer et al. 2017), red deer (Martin et al. 2009; Balachandran et al. 2010), sika deer (*Cervus nippon*) (Sohn et al. 2020) and reindeer (Mitchell et al. 2012). As further evidence to support the efficacy of oral infection and to track early CWD pathogenesis, an experimental study (Sigurdson et al. 1999) was conducted employing mule deer fawns captured outside the CWD endemic region (Miller et al. 2000; Miller and Williams 2003). The fawns were orally exposed to pooled brain homogenate harvested from naturally exposed CWD-infected mule deer. Conventional CWD detection methods (IHC) were used to demonstrate PrP^{CWD} deposition within the retropharyngeal node, Peyer's patches and ileocaecal node as early as 1.5 months post-inoculation (mpi), and within tonsil as early as 2.75 mpi. These data were critical for providing: (i) proof of concept for CWD infection via oral exposure, and (ii) broad dissemination in lymphoid tissues adjacent to mucosal surfaces early in infection suggesting the possibility of prion shedding in bodily excretions (saliva, urine, feces).

A subsequent experimental study in mule deer using the same source inoculum, route of inoculation and CWD detection methodology as the above mule deer fawn study (Sigurdson et al. 1999) permitted clinical disease and PrP^{CWD} dissemination monitoring throughout the disease course (inoculation to terminal clinical disease) (Fox et al. 2006). This study resulted in clinical manifestation consistent with CWD disease between 20 and 26 months post-oral exposure. Clinical presentation including dullness in eyes, diminished alertness, misdirected behaviors and piloerection occurred as early as 14–19 mpi. As clinical disease progressed, additional behavioral changes were noted. These changes included blank staring, uncharacteristic or subdued

Fig. 1 Distribution of Chronic Wasting Disease in North America and Scandinavia. **A.** Distribution of Chronic Wasting Disease in North America. Credit and Source: Dr. Bryan Richards; National Wildlife Health Center, United States Geological Survey, Distribution of Chronic Wasting Disease in North America, <https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0>,

January 2022. **B.** Distribution of Chronic Wasting Disease in Norway, Sweden and Finland. Credit and Source: Dr. Christer Rolandsen; The Norwegian information portal for deer management (www.hjortevilt.no) / Norwegian institute for nature research (www.nina.no). , October 26, 2021

responses to aversive stimuli, lowered head, ataxia and inefficient grazing. With time, loss of body condition became more pronounced. Excessive thirst (polydipsia), urination (polyuria) and saliva generation were described for some deer late in disease course. PrP^{CWD} was detected within a broad array of lymphoid tissues between 3 and 21 mpi. In spleen tissue, PrP^{CWD} deposition was first demonstrated at ~6 mpi. Detection was limited in thymus, GALT of colon and esophagus or lymphoid aggregates of the soft palate, lung and renal pelvis. Earliest PrP^{CWD} detection within the myenteric and submucosal plexuses was reported at ~9 mpi, with detection within tissues of the central nervous system (CNS) as early as ~6 mpi. Deposition of PrP^{CWD} was shown in thoracic and lumbar spinal cord, and cord/medulla junction at ~9 mpi with more widespread CNS and neuroendocrine

tissue involvement revealed at ~16 mpi. These findings provided: (i) evidence that oral dosing resulted in similar timing and clinical presentation as described for natural CWD infections, (ii) refined detailing of early clinical disease manifestation and staging of clinical course, and (iii) evidence for and timing of PrP^{CWD} distribution in a broad array of tissues.

Further exploration into how CWD is transmitted and sustained among native cervid populations was performed by a series of experimental studies conducted in cohorts of hand-raised and indoor adapted white-tailed deer (WTD). White-tailed deer were sourced from a region of the USA that to date is free of CWD (Georgia, USA). The controlled nature of housing permitted assessment of point source inocula, and temporal detection of CWD in serially collected

B **Fig. 1** (continued)

lymphoid biopsies and bodily excretions. This native host model provided a previously untenable level of longitudinal monitoring and evaluation of CWD transmission, prion dissemination and shedding.

Taking cues from natural infection observations (Miller et al. 2004), this WTD model was employed to assess the presence of infectivity in several bodily fluids and excretions (secreta)—saliva, urine, feces and blood. Although excretions were known to harbor infectious particles, it remained undefined which (saliva, urine, feces, blood) contained sufficient infectivity to initiate CWD infection. Cohorts of WTD

were maintained in separate suites, permitting assessment of the infectious potential of saliva, urine, feces or blood (Mathiason et al. 2006). The inoculum was collected from clinical CWD positive naturally infected mule deer. Separate cohorts of WTD were orally dosed with saliva (50 ml), or a combination of urine & feces (50 ml or gr/each), or received a blood transfusion (250 ml). As controls for infection, a cohort of WTD received orally dosed CWD positive brain (10 gr), and a cohort of WTD was orally dosed with brain, saliva, urine, feces and blood transfusion from confirmed CWD-negative WTD (same amounts and dosing as

per above). Deer were monitored weekly for clinical signs of disease. Serial lymphoid (tonsillar and RAMALT) biopsies were taken at 3 month intervals to assess for the presence of PrP^{CWD} by IHC and Western blot analysis. Saliva, urine, feces and blood were collected at the same time interval, with each aliquoted and stored at -80C. The first lymphoid PrP^{CWD} positivity was noted at 3 mpi in WTD orally dosed with brain tissue; with all animals becoming lymphoid biopsy positive by 12 mpi. Animals inoculated with saliva or blood transfusion became lymphoid biopsy positive at 12 mpi, with demonstrable lymphoid PrP^{CWD} deposition in all deer by 19 mpi. A subset of saliva and blood-inoculated WTD were further monitored for clinical disease onset. First signs consistent with CWD clinical presentation were identified between 15 and 19 mpi (staring, increased presence of whites of eye, behavioral changes) with noted progressive development (wide-leg stance, piloerection, polydipsia, polyuria) to terminal clinical presentation (leg lifting, head and neck arching) occurring between 24 and 33 mpi. None of the WTD orally inoculated with urine and feces resulted in PrP^{CWD} detection in lymphoid tissue by conventional assay (IHC and Western blot) throughout the 19 month monitoring period. Interestingly, brain tissue harvested from this cohort was later evaluated by a sensitive mouse bioassay (Tg(CerPrP-E226)5037 (Angers et al. 2009)). Assessment revealed the presence of CWD infectivity (Haley et al. 2009). A follow-up bioassay study in the native host model was conducted to determine the presence of CWD infectivity in secreted shed by asymptomatic lymphoid biopsy positive WTD. The serial sample collections of saliva, urine, feces and blood harvested from deer in the initial study served as the point source inoculum for this second study (Mathiason et al. 2009). This set of WTD cohorts was inoculated as per the initial study. Interestingly, the saliva and blood harvested from the subclinical animals demonstrated similar time to lymphoid biopsy positivity as compared to the group that received saliva and blood from CWD-clinical animals. This suggests the duration of infectious shedding may not be confined to animals with obvious symptomatic infections. To complement these WTD studies to assess indirect transmission of CWD in the absence of direct animal-animal contact, a cohort of deer was exposed to bedding, water, and feed buckets (i.e., fomites) from suites containing CWD-lymphoid biopsy positive WTD (Mathiason et al. 2009). This cohort, exposed to CWD solely by indirect environmental exposure, demonstrated lymphoid PrP^{CWD} deposition between 15 and 19 mpi. This study underlines the importance of the presence of maintained infectivity shed with secretions to the environment, and how they may play a role in the natural transmission of CWD. Taken together, these studies revealed that: (i) sufficient infectivity was present within brain, saliva and blood collected at clinical and preclinical stages of disease to initiate CWD infection that manifest in clinical TSE disease presentation similar to that

observed in natural cervid infections (Miller et al. 2004; Mathiason et al. 2006), (ii) oral exposure to large volumes of urine and feces from CWD-infected cervids did not result in PrP^{CWD} deposition or clinical manifestation at 19 mpi (Mathiason et al. 2009), rather, yielded asymptomatic CWD infections (Haley et al. 2009), and (iii) that sufficient infectivity was present in fomites (hay, bedding, buckets, water) from CWD-infected WTD suites to initiate indirect (no direct physical contact) asymptomatic infections between 15 and 19 mpi (Mathiason et al. 2009).

With the infectious potential of secreted demonstrated, PrP^{CWD} burden in oropharyngeal, urogenital and gastrointestinal tissue sources of excreta were evaluated. These tissues were evaluated by **protein misfolding cyclic amplification (PMCA)**, an assay that allows amplification and detection of prion seeding activity in tissues harboring low concentrations of CWD (Saa et al. 2005). Low prion burdens were found in salivary gland, urinary bladder and distal intestinal tract tissues (Haley et al. 2011). These findings offer insights into the transmission dynamics of CWD as well as intra-host trafficking. Further insights examining early pathways of intra-host prion spread demonstrated PrP^{CWD} and amyloid seeding activity in oropharyngeal lymphoid tissues as early as 1-month post-oral inoculation with PrP^{CWD} levels present at 4 mpi approaching levels observed in terminal disease (Hoover et al. 2014). This study utilized another recent advancement in the detection of low prion burdens, **real-time quaking induced conversion (RT-QuIC)** (Atarashi et al. 2008). These results support early CWD replication in tissues that produce saliva that may contribute to shed prions in excreta. Together these WTD studies support the presence of amyloid seeding activity (RT-QuIC and PMCA) in serial collections of excreta (urine, saliva and feces) harvested across the longitudinal course of CWD infection, suggesting that CWD prions are intermittently shed in excreta during the full course of disease (Henderson et al. 2015a, 2017; Davenport et al. 2017; Tennant et al. 2020). These findings support earlier demonstration of the infectious agent in feces and saliva of CWD-infected asymptomatic free-ranging cervids (Tamguney et al. 2009, 2012).

The dissemination of CWD seeding activity to excretory tissues suggested robust intra-host CWD trafficking that remained understudied. Given the robust infectivity observed in WTD that received blood via transfusion, the role of hematogenous-borne prions was evaluated. Blood is a mixture of cellular and acellular components, and additional bioassay studies were conducted in WTD to evaluate which may harbor the infectious CWD agent. These studies indicated that CWD blood infectivity was associated with the cellular fraction of blood, with a significant role for B cells and platelets in trafficking CWD infectivity in vivo (Mathiason et al. 2010). These findings support earlier tissue-based studies associating putative follicular B

cells with PrP^{CWD} (Sigurdson et al. 1999). Taking advantage of the advances made in methods for in vitro detection of CWD seeding activity in blood, it was revealed that CWD prions cross the mucosal surface as early as 15 min post-oral exposure (Elder et al. 2013; McNulty et al. 2020). This was followed by an eclipse phase and subsequent bi-motile presence of amyloid seeding activity between 2 and 3 mpi and again near lymphoid biopsy positivity (McNulty et al. 2020; Elder et al. 2015). These findings suggest: (i) roles for specific blood cell subsets in prionemia, (ii) that hematogenous prions peripheralize early in infection extending to all parts of the body, which may result in (iii) new routes of neuroinvasion.

CWD infectious dose and route of exposure

This WTD experimental model has provided key insights into the spread of CWD in and between susceptible cervids. It has also identified likely sources for shed infectivity. Due to the tightly controlled housing and longitudinal monitoring developed with this model, it is also uniquely relevant to evaluate the minimum amount of peripherally shed infectious agent required to propagate CWD infection. Furthermore, this experimental model provided opportunity to assess whether acquiring peripherally shed prions, by single bolus or multiple low dose exposures, are important factors in CWD transmission. One study incorporated very low doses of saliva or brain (300 ng) in single- and multiple-dose regimens (Denkers et al. 2020). Oral exposures of brain or saliva—dosed as a single 300 ng bolus, three 100 ng doses, or ten 30 ng doses, were delivered orally over 12 weeks. WTD cohorts receiving the single 300 ng bolus and three 100 ng dose became infected, while WTD cohorts dosed with ten 30 ng doses remained negative. This study revealed: (i) that the minimum infectious CWD dose approximates 100–300 ng CWD positive brain or saliva equivalent, (ii) CWD infection appears to conform more with a threshold rather than cumulative dose dynamic, and (iii) that while time to first detection of infection was prolonged in low dose recipients, once infection was established no differences in disease pathogenesis were observed to higher dosed WTD.

The body of work conducted in natural and experimental settings clearly demonstrates that cervids are susceptible to oral CWD exposures, whether by direct (animal-to-animal) or indirect (environmental) contact with the infectious agent. Regardless of source and route, transmission of CWD prions via contact with the alimentary and/or respiratory mucous membranes seems assured. A study to determine the efficacy of respiratory mucosa exposure via aerosolization was undertaken in the WTD experimental system. It was found that this is an efficient mode of transmission (5 mg brain tissue exposure), with infection kinetics equal to oral exposure

(first biopsy positive at 3 mpi, with clinical presentation in all deer at 12 mpi) (Denkers et al. 2013). An additional study was conducted in WTD sourced from Missouri, USA, that were housed indoors (Nichols et al. 2013). The deer were inoculated by intranasal expulsion of lyophilized CWD positive brain (200 mg) bound to montmorillonite clay (250 mg). The study revealed that detectable PrP^{CWD} deposition was present within lymphoid tissues as early as ~3 mpi.

The housed WTD model of CWD has allowed for greater understanding of prion transmission, dissemination and shedding. Important facets of CWD pathology such as minimum infectious dose and alternative routes of infection continue to be explored. In addition to these important questions, this model has provided greater insight into other areas of CWD research such as: (i) CWD detection sensitivity (Haley et al. 2012; Henderson et al. 2013, 2020; Denkers et al. 2016), (ii) semi-quantitation of CWD loads (Henderson et al. 2015b), (iii) longitudinal assessments of CWD presence in saliva, urine, feces and alimentary tract, and third eyelid (Henderson et al. 2015a, 2017; Tennant et al. 2020; Cooper et al. 2019), (iv) the ability to use paraffin embedded tissues for the detection of CWD prions (Hoover et al. 2016) and, (v) direct comparison of CWD conventional, amplification and bioassay methods (McNulty et al. 2019).

CWD vertical transmission

Horizontal CWD transmission, whether by direct animal-to-animal contact, or indirect contact with prion infectivity shed into the environment, has been well documented. Fewer studies have monitored the possibility of vertical transmission from mother-to-offspring. A small cervid model, the Reeves' muntjac deer, provided opportunity to assess timed pregnancies in dams with known CWD status. This cervid model is polyestrous, indoor maintainable, and has similar CWD susceptibility and pathogenesis as native mule deer and WTD (Nalls et al. 2013). Muntjac dams were orally or subcutaneously inoculated with CWD positive brain tissue, becoming lymphoid biopsy positive between 3 and 4 mpi. Naïve and CWD positive dams were bred by live coverage with naïve muntjac bucks. Fawns were live-birthed, or fetal tissues were harvested at specific time points across gestation (1st, 2nd and 3rd trimester). The studies demonstrated CWD transmission from mother-to-offspring leading to detection of PrP^{CWD} deposition in lymphoid tissue of fawns born to CWD-infected dams as early as 40 days post-birth that resulted in progressive clinical CWD disease between 30 and 54 months post-birth (Nalls et al. 2013). PMCA seeding activity was present in fetuses as early as the first trimester of pregnancy (Nalls et al. 2013), with infectivity (demonstrated by Tg(CerPrP-E226)5037[±] mouse bioassay)

present in the pregnancy microenvironment (uterus, amniotic fluid and placentomes) of CWD-infected pre-symptomatic dams (Nalls et al. 2017). To determine the impact, mother-to-offspring transmission may play in native free-ranging cervid populations in utero harvested fetal samples collected from elk and WTD in CWD-endemic areas were assessed for CWD by PMCA and RT-QuIC amplification assays. The presence of prion seeds was demonstrated in fetal tissues from free-ranging elk (Selariu et al. 2015) and WTD (Nalls et al. 2021; Bravo-Risi et al. 2021), as well as in male reproductive tissues and semen of WTD (Kramm et al. 2019) providing evidence that vertical and/or sexual transmission may play a role in the facile transmission of CWD.

Genetic factors that influence CWD susceptibility

The above outlines factors involved in the spread of CWD from animal to animal, both directly and indirectly. Yet, another important facet in CWD transmission is the susceptibility of the naïve host to acquire the disease. A host's *Prnp* genotype and the primary structure of the PrP^C protein(s) encoded have been shown to contribute to varying degrees of susceptibility to species-specific prion disease, in some cases completely preventing infection. In free-ranging cervid populations, the prevalence of CWD is lower in populations expressing at least one copy of specific *Prnp* polymorphisms suggesting reduced or delayed susceptibility to infection (Robinson et al. 2012; Monello et al. 2017; Haley et al. 2019). Understanding the prevalence of these susceptible and resistant *Prnp* genotypes in free-ranging cervid populations would allow for greater understanding of transmission dynamics and susceptibility compared to cervid herds with known CWD prevalence (Arifin et al. 2020). Experimental studies conducted in the native host have provided improved understanding of the role these genotypes play in disease. To test the hypothesis that *Prnp* genotype affects CWD disease progression and susceptibility in WTD, an experimental oral infection was performed (Johnson et al. 2011). Deer fawns possessing *Prnp* polymorphisms H95 and S96 were orally inoculated to determine the genetic contribution on the rate of disease progression. The authors demonstrated that deer homozygous for the wild-type prion protein Q95G96 succumbed to CWD infection significantly earlier than deer heterozygous or homozygous for the H95/S96 prion allele. Similarly, oral infection of Rocky Mountain elk (Hamir et al. 2006) revealed that elk expressing *Prnp* codon 132MM or 132LM were susceptible to oral inoculation (23–40 mpi), while those expressing codon 132LL elk remained clinically normal for 4+ years, developing clinical CWD disease between 59 and 64 mpi. This suggested that 132LL elk may have reduced susceptibility or prolonged incubation to

CWD infection. Further analysis of brain tissues from this study revealed differences in PrP^{CWD} deposition location and abundance. Elk harboring the susceptible genotype showed greater prion burden and neuropil deposition. In contrast, elk with reduced susceptible genotype showed reduced burden and glial associated deposition. These findings demonstrate that elk with longer incubation times (132LL) carried lower PrP^{CWD} burdens than those that progressed to terminal clinical presentation in a shorter timeframe (132MM or 132LM) (Moore et al. 2018).

Enhanced understanding of *Prnp* polymorphisms represents a powerful tool to combat TSEs, with a clear demonstration observed in the sheep prion disease, scrapie. Sheep flock improvement programs have successfully reduced the cases of classical scrapie by incorporating breeding strategies to select for *Prnp* resistant stock (Soto et al. 2021; Hagenaars et al. 2010). Experimental studies and field studies that continue to shed light on the presence of *Prnp* polymorphisms and associate CWD status will help guide efforts to manage CWD through selective breeding strategies (Haley et al. 2021).

CWD vaccine development

Understanding factors that drive transmission and intra-host dissemination are key to understanding CWD disease and how to potentially stem its spread. Currently, there is no vaccine or treatment for any prion disease, including CWD. The dilemma with immune system-mediated vaccine approaches is that prion diseases do not elicit a *bona fide* immune reaction since PrP^C and PrP^{Sc} have the same primary structure (Aguzzi 2003; Aucouturier and Carnaud 2002; Mabbott et al. 2018). Newly generated prions are recognized as self-proteins by the immune system, and as adaptive immunity is mainly based on linear epitopes, prion infections do not result in detectable humoral and cellular immune responses. As well, there is no evidence that PrP^{Sc} represents a pathogen-associated molecular pattern for innate immunity, although microglia involvement has been described (Mabbott et al. 2018; Perry 2004). Nevertheless, it has been demonstrated that immunization approaches can be effective against prion infections (Enari et al. 2001; Peretz et al. 2001; Heppner et al. 2001; White et al. 2003).

Novel vaccines have been evaluated in three cervid species, two with CWD challenge. A recombinant prion protein vaccine (Abdelaziz et al. 2018) was assessed in reindeer to determine if homologous and aggregation-prone recombinant prion protein (PrP) could overcome self-tolerance and induce autoantibody production against the cellular isoform of PrP^C. Reindeer were dosed three times with 500 ug of Mouse dimer (Mdi) or Deer dimer (Ddi) immunogen with 5uM CpG adjuvant via subcutaneous route. This pilot study

resulted in appreciable humoral immune responses in reindeer vaccinated with Mdi or Ddi recombinant protein, and post-immune sera harvested from reindeer receiving the Ddi vaccination mitigated CWD propagation in cell culture. Further studies in a cervid challenge model will be necessary to determine if this vaccine is efficacious in the control of CWD. A second nonchallenge CWD vaccine platform was investigated in WTD. Here, the authors targeted epitopes whose exposure for antibody binding depends on disease-associated misfolding of PrP^C into PrP^{Sc} (Taschuk et al. 2017). Their vaccine consisted of a replication-incompetent human adenovirus expressing a truncated rabies glycoprotein G recombinant fusion with the rigid loop epitope (hAd5:tgG-RL). Oral immunization led to the induction of PrP^{Sc} specific systemic and mucosal antibody responses. Again, further studies in a cervid CWD challenge model will be necessary. A third vaccine targeting a YYR disease-specific epitope was evaluated in elk with natural exposure to CWD contaminated environment (Wood et al. 2018). Elk were dosed intramuscularly with 50 µg of the recombinant YYR-Lkt fusion protein with Emulsigen D at day 0, day 42, and annually for three years. All vaccinated elk produced YYR-specific antibody titers. Yet, the mean survival time was significantly shorter for vaccinated (800 days) vs unvaccinated elk (1062 days) after they were exposed to CWD contaminated paddocks. A fourth vaccine incorporated mucosal exposure to an attenuated *Salmonella* expressing PrP in white-tailed deer (Goni et al. 2015). Once a mucosal response was established, the vaccinated deer were boosted orally and locally by application of polymerized recombinant PrP onto tonsils and rectal mucosa. The white-tailed deer were then challenged orally with CWD. A significant prolongation of the incubation period was noted in vaccinated (909 dpi) vs controls (602 dpi) with one (1/5) deer remaining CWD free both clinically and by lymphoid biopsy. Efforts to find an effective vaccination strategy for CWD may require combined vaccine approaches and the incorporation of low dose or natural challenge longitudinal cervid model studies to assess their efficacy.

The current promising approaches in CWD vaccinology employ insights into the structure of PrP^{Sc} (Wille and Requena 2018), and the proof-of-concept that tolerance to PrP^C can be overcome by active vaccination as described above (Abdelaziz et al. 2018). Analysis of PrP^{Sc} allowed definition of structure-specific surface epitopes as vaccine targets (Fang et al. 2019, in preparation). Immunization with this rationally designed, structure-based antigen, generated immune responses that specifically recognized PrP^{Sc} in brain homogenates from prion-infected animals and protects transgenic mice carrying different human *PRNP* mutations against their disease-causing effects (Fang et al. in preparation). The finding that tolerance to PrP^C can be overcome by active vaccination in reindeer and in mouse models, resulting

in robust humoral and cellular responses against PrP without inducing unwanted side effects (Abdelaziz et al. 2017, 2018; Gilch et al. 2003; Polymenidou et al. 2004; Kaiser-Schulz et al. 2007) provides a second promising avenue to an efficacious CWD vaccine. Induced anti-PrP antibodies were found to block prion infection in vitro and resulted in prolonged survival of CWD-infected transgenic mice by up to 70% (Abdelaziz et al. 2017, 2018). The accumulated preliminary findings from these two diverse vaccine approaches indicate feasibility, efficacy and safety for longitudinal assessment, singly and in combination, in the experimental native white-tailed deer system described earlier in this review.

Summary

Over two decades of experimental studies in the native, cervid host has provided new insights to CWD pathogenesis, transmission dynamics, the importance of *Prnp* polymorphisms in infection susceptibility and disease progression, and the development of CWD mitigation strategies. Furthermore, use of these model systems has resulted in improved CWD detection methodologies, allowing greater exploration of key mechanistic and societal impacts associated with prions; including the prion conversion event and strain development, the impacts CWD has on free-ranging cervid populations, herd management, hunting and cervid industries, and the potential of transspecies transmission events to species sympatric with cervids, including humans.

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Declarations

Conflict of interest The corresponding author states that there is no conflict of interest.

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