The ecology of chronic wasting disease in wildlife

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ABSTRACT

Prions are misfolded infectious proteins responsible for a group of fatal neurodegenerative diseases termed transmissible spongiform encephalopathy or prion diseases. Chronic Wasting Disease (CWD) is the prion disease with the highest spillover potential, affecting at least seven Cervidae (deer) species. The zoonotic potential of CWD is inconclusive and cannot be ruled out. A risk of infection for other domestic and wildlife species is also plausible. Here, we review the current status of the knowledge with respect to CWD ecology in wildlife. Our current understanding of the geographic distribution of CWD lacks spatial and temporal detail, does not consider the biogeography of infectious diseases, and is largely biased by sampling based on hunters’ cooperation and funding available for each region. Limitations of the methods used for data collection suggest that the extent and prevalence of CWD in wildlife is underestimated. If the zoonotic potential of CWD is confirmed in the short term, as suggested by recent results obtained in experimental animal models, there will be limited accurate epidemiological data to inform public health. Research gaps in CWD prion ecology include the need to identify specific biological characteristics of potential CWD reservoir species that better explain susceptibility to spillover, landscape and climate configurations that are suitable for CWD transmission, and the magnitude of sampling bias in our current understanding of CWD distribution and risk. Addressing these research gaps will help anticipate novel areas and species where CWD spillover is expected, which will inform control strategies. From an ecological perspective, control strategies could include assessing restoration of natural predators of CWD reservoirs, ultrasensitive CWD detection in biotic and abiotic reservoirs, and deer density and landscape modification to reduce CWD spread and prevalence.

Key words: Cervidae, Chronic Wasting Disease, CWD, prions, reservoirs, spread, wildlife, zoonotic.

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I. INTRODUCTION

Prions (see Table 1) are the proteinaceous infectious agents responsible for human and animal prion diseases (Prusiner, 1982). Prions are composed of a misfolded aggregated form of the prion protein (termed PrP Sc) that is able to template the conversion of the natively folded prion protein (PrP C) through a seeding mechanism resulting in the formation of large amyloid-like fibrillar aggregates that accumulate in the brain of infected hosts (Prusiner, 1982; Soto, 2012). The chain reaction of prion replication leads to the accumulation of toxic structures, resulting in progressive neurodegeneration, and is invariably fatal to the host (Prusiner, 1982). Five prion diseases are currently recognized in humans (Creutzfeldt-Jakob Disease, Variant Creutzfeldt-Jakob Disease, Gerstmann-Strassler-Scheinker Syndrome, Fatal Familial Insomnia, and Kuru) and seven in animals (Bovine Spongiform Encephalopathy, Ungulate Spongiform Encephalopathy, Scrapie, Transmissible mink Encephalopathy, Camel Prion Disease, Feline Spongiform Encephalopathy, and Chronic Wasting Disease, or CWD) (Johnson, 2005; Babelhadj et al., 2018; CDC, 2018b).

II. THE BIOLOGY OF PRIONS AND PRION REPLICATION

The main event in prion disease is the conversion of the natively folded PrP C into the misfolded, toxic, and infectious PrP Sc, which generates a neurodegenerative disease expressed histopathologically as spongiform encephalopathy (Soto & Satani, 2011). However, the molecular basis of these disorders and the factors that trigger the protein misfolding and initiate the pathology remain unclear (Prusiner, 1998). In humans, genetic forms of prion disease are linked to mutations in the gene encoding for the prion protein (Prnp), which likely induce the misfolding and aggregation process, increasing rates of prion replication. On the other hand, acquired forms of prion diseases in humans and animals are caused by exposure to infectious PrP Sc (e.g. iatrogenic infection).

In humans, the most prevalent form of the disease has a sporadic origin, i.e. unknown etiology. Various hypotheses have been proposed to explain the formation of the first prion molecules in sporadic prion diseases (Safar, 2012). One possibility is that somatic mutations or errors in protein synthesis may initiate the chain reaction of protein misfolding. Other possibilities are stochastic changes to the structure of PrP C or a failure in the biological clearance of misfolded proteins to eliminate low levels of PrP Sc produced continuously during life. Fluctuations in the environment (e.g. changes in pH, salinity, temperature) could influence the persistence of infectious prions in the landscape (Bartelt-Hunt & Bartz, 2013), so these changes may also enhance spontaneous protein misfolding and prion replication, but this possibility has not been explored. Another hypothesis suggests that prion formation might be promoted or accelerated by traumatic brain injury, which potentially damages tissues and/or axons facilitating metabolic, ionic, and cytoskeletal damage, or causes transcriptional errors that trigger improper folding and clumping of proteins inside nerve cells in the brain (e.g. neurons or astrocytes), as observed for tau protein (Woerman et al., 2016; Rubenstein et al., 2017; Edwards et al., 2019).

In wildlife, chronic trauma could come from agonistic behaviour of males from the families Cervidae (deer) and Bovidae (cattle and sheep) during dominance displays, in which heads are used as weapons during fighting (i.e. ‘rutting’; Barrette, 1977). Chronic trauma behaviour is exhausting, damaging, and potentially lethal (Wilkinson & Shank, 1976; Barrette, 1977; Leslie & Jenkins, 1985; Geist, 1986; de Vos, Broxk & Geist, 2016). Chronic trauma has not been studied in the context of CWD in animals. However, this phenomenon could mirror the effects of traumatic brain injury in human neurodegenerative diseases, such as chronic traumatic encephalopathy produced by the accumulation of misfolded aggregates composed of the tau protein (Woerman et al., 2016; Rubenstein et al., 2017; Edwards et al., 2019). Thus, chronic trauma may have played a role in the origin of CWD and in recent ‘spontaneous’ CWD reports in Scandinavia (Benestad et al., 2016; Evira, 2018; Vikøren et al., 2019), although this remains speculative and further research is required.

An important characteristic of the prion agent is its ability to infect some species and not others. This phenomenon is known as the species barrier (Hill & Collinge, 2004; Moore, Vorberg & Priola, 2005). The species barrier in prion disease is mostly controlled by similarity in the PrP sequence between the donor and receptor species, but it is also known to be dependent on the strain features of the infectious material, which are dependent on structural differences in infectious PrP Sc (Hill & Collinge, 2004; Moore et al., 2005). Even between phylogenetically close species...
III. CHRONIC WASTING DISEASE BIOLOGY, EPIDEMIOLOGY, AND TRANSMISSION

(1) CWD in animals

Chronic Wasting Disease (CWD) is the most worrisome member of the group of prion diseases, because it affects wildlife, and is currently spreading rapidly in North America and has recently been detected in Europe (Fig. 1). However, our knowledge of the origins of CWD is limited. The first observation of CWD occurred in 1967 in a captive deer facility in Colorado (Williams & Young, 1980). There is speculation that CWD emerged in this facility due to scrapie spillover from sheep co-housed there (Blumhardt, 2018). However this has not been confirmed. Instead, this early detection could be related to intense observation by veterinarians in this research facility. More historical–epidemiology research is necessary regarding CWD in Colorado to understand better the apparently spontaneous CWD cases recently confirmed in Europe (Benestad et al., 2016; Evira, 2018; Vikøren et al., 2019).

Experimental challenges using infected brain homogenate of scrapie-infected sheep suggest that scrapie prions from sheep can infect elk (*Cervus elaphus nelsoni*) (Hamir et al., 2004) and white-tailed deer (*Odocoileus virginianus*) (Greenlee, Smith & Kunkle, 2011). However, to date, scrapie transmission to cervids has only been documented generally using infectious routes that are not epidemiologically relevant (e.g. intra-cranial inoculation). CWD was the first, and continues to be the clearest, example of a transmissible prion among free-ranging wildlife. Experiments and observations demonstrate that CWD prion transmission can occur vertically (e.g. mother to offspring) (Selariu et al., 2015) or horizontally (e.g. direct animal contact, environmental...
Fig. 1. Geographic distribution of Chronic Wasting Disease (CWD) reports. (A) The region with the most cases and areas infected with CWD is North America; (B) Europe has reported CWD in Norway, Sweden, and Finland; (C) Asia reported CWD in South Korea. Red: counties (in USA) and wildlife management areas (in Canada) with reports of CWD in wild cervids; dark grey: states/provinces reporting CWD in captive cervids; light grey: states with CWD detection in wild cervids; white: areas with no reports of CWD; (D) Timeline denoting the first detections of CWD in specified regions for each country. Data derived from CDC (2019) and CWD Alliance (2019).

infection) (Mathiason et al., 2009; Denkers et al., 2013; Zabel & Ortega, 2017) (Fig. 2). In North America, species known to be susceptible to natural infection include elk, white-tailed deer, mule deer (Odocoileus hemionus), black-tailed deer (O. h. columbianus), and moose (Alces alces), and the introduced red deer (C. elaphus) (Williams & Young, 1980; Spraker et al., 1997; Baeten et al., 2007) (Fig. 3). Europe has documented transmission in free-ranging reindeer (also known as caribou; Rangifer tarandus), red deer, and moose in Norway, Sweden, and Finland (Benestad et al., 2016; Evira, 2018; Vikeren et al., 2019). South Korea has reported CWD in captive elk transferred from a Canadian captive cervid facility (Kim et al., 2005). After infection, incubation period in wild cervids is generally between 2 and 4 years with a minimum of 16 months before development of symptoms (Williams, 2005). During the pre-clinical, asymptomatic phase, prions can be detected in faeces, urine, and saliva as early as 6 months post-infection (Plummer et al., 2017). Symptoms associated with late-stage CWD infection include emaciation, excessive salivation, behavioural changes, ataxia, depression, and weakness (Williams & Young, 1980; Spraker et al., 1997).

The USA has the most widespread CWD infection in the world (Fig. 1). As of August 2019, the USA had confirmed CWD in 26 states, including in free-ranging cervids in 279 counties in 24 states (CDC, 2019) and in captive deer in 17 states (USGS, 2019). In some of these localities, CWD prevalence in wild white-tailed deer reached as high as 40% in adult females and 50% in adult males (Edmunds et al., 2016; Carbon et al., 2018). The highest infection rate has usually been found in older males followed by
Ecology of CWD

Fig. 2. Role for intermediate species and environmental reservoirs in the spread of Chronic Wasting Disease (CWD). Wild cervid populations serve as the reservoirs of CWD (Carlson et al., 2018), acting as source for spillover to other species and the environment. Natural spread: spread in natural areas associated with high CWD prevalence and dispersal observed in male white-tailed deer (Clements et al., 2011; Carlson et al., 2018). Unknown susceptible: species that have shown successful PrPSc infection under experimental settings, but for which no evidence is available under natural conditions; potentially susceptible predators (e.g. coyotes) and scavengers (e.g. crows and raccoons) exist that could act as vectors of the infectious prion (Bunk, 2004; Hamir et al., 2007; Fischer et al., 2013; Moore et al., 2019). Similarly, while fawns are known to be susceptible, little is known of their role in the shedding and spread of CWD. Known susceptible: species known to be susceptible to CWD infection, including mule deer, black-tailed deer, elk, white-tailed deer, red deer, moose, and reindeer (caribou) (Williams & Young, 1980; Spraker et al., 1997; Baeten et al., 2007; Benestad et al., 2016; Evira, 2018). Species susceptible in laboratory experiments include Reeve’s muntjac and fallow deer. Anthropogenic spread: spread of CWD facilitated by human intervention, including translocation of infected deer (e.g. deer farms, carcasses). Environmental reservoirs: infected fluids or tissues (e.g. urine, saliva, faeces, blood) deposited in the environment (e.g. water, grass, soil, rocks) remaining infectious for months, years, or decades. Black arrows, observed in the wild or in laboratory conditions; red arrows, uncertain, requiring additional research. Older females, and yearling males (Heisey et al., 2010), but not in all areas (Edmunds et al., 2016). Older males may be at the highest risk due to their broader home range, which increases their chance of interacting with infected deer or contaminated landscapes, their less-cohesive social structuring than in females, and their higher contact during the mating season (e.g. fights with other males, allogrooming courtship, copulation, scent verification). In North American elk and deer species, males disperse (permanent movement away from a natal range) more frequently than females (Skuldt, Mathews & Oyer, 2008; Clements et al., 2011; Miller & Conner, 2013; Nobert et al., 2016) (Fig. 2). However, other movement behaviours such as migration (primarily in western North America) (Conner & Miller, 2004; Farnsworth et al., 2006) and exploratory movements of young individuals (Oyer, Mathews & Skuldt, 2007) also potentially impact the spatial spread of prions without being linked to dispersal behaviours. Indeed, CWD infection itself could impact prion spread: CWD causes increased activity (hyperexcitability) in the early stages of infection and a search for drinking water in the late clinical stages (e.g. insipidus-like syndrome of polydipsia) (Williams & Young, 1993; Miller, Wild & Williams, 1998). Additionally, infected deer reduce their spatial movements late in infection due to the diminished alertness, movement, and lethargy observed in the clinical phase of the disease (Fox et al., 2006; Edmunds et al., 2018). Reduced spatial movement during the last stage of the infection may facilitate concentration of infectious material in specific locations.

(2) CWD and public health

The potential for CWD to infect humans is highly controversial, but the general consensus is that transmission to humans cannot be entirely ruled out based on current
Fig. 3. Taxonomic breadth of Chronic Wasting Disease (CWD) infections in the Cervidae. Cladogram denotes cervid species found naturally susceptible (red), susceptible under experimental inoculation (blue), and of unknown susceptibility (black). Figure modified from Gilbert et al. (2006), based on species classification from the Integrated Taxonomic Information System (www.itis.gov) and sequences available from Genbank (https://www.ncbi.nlm.nih.gov/genbank/). Deer illustrations from Lydekker (1898).

evidence (Waddell et al., 2018). Experiments using transgenic mouse models have shown negative results for zoonotic risk (Kong et al., 2005; Tamgney et al., 2006; Sandberg et al., 2010). Using in vitro conversion studies, it was found that CWD prions can replicate at the expense of the human protein, but only after the CWD strain has been adapted by various rounds of replication in deer (Barria et al., 2011), suggesting that under certain conditions CWD may threaten human health. Notably, CWD was shown to transmit to various species of non-human primates (Marsh et al., 2005). Of particular note, recent studies report transmission of CWD to macaques (Macaca fascicularis) even by oral administration of brain and muscle tissues (Czub et al., 2017) but these results have been questioned (Race et al., 2018). The USA Centers for Disease Control and Prevention (CDC) recommend that people who harvest deer from CWD-affected areas test their deer or elk for CWD and do not consume venison from a known CWD-positive cervid (CDC, 2018a).

A recent in vitro assessment showed that CWD zoonotic potential is affected by factors such as PrPSc strain, cervid species, and geographical location from which CWD originates. In one study, PrPSc from white-tailed deer did not show zoonotic potential, while PrPSc from elk and reindeer were compatible with the human protein (Barria et al., 2018), supporting the idea of strain-specific risk for human CWD infection that has yet to be considered in zoonotic risk assessments. Transmission of CWD from wildlife to cattle has been observed only under experimental conditions (Hamir et al., 2001, 2005). Neither livestock nor humans have developed the disease, even after residing in CWD-endemic areas for decades. A renewed concern is that the CWD situation mirrors the early stages of BSE research, in which a limited number of species was thought to be susceptible to infection (e.g. mice, hamsters, and primates) (Osterholm et al., 2019). At that time, it was thought that scrapie-associated prions causing BSE would not infect people based on the fact that people ate scrapie-infected meat for centuries with no evidence of infection (Bunk, 2004). It was discovered later that BSE is able to cross the species barrier and infect humans after oral exposure (Johnson, 2005). Changes in prion composition and virulence occur during passages through different species (Raymond et al., 2007; Race et al., 2018), suggesting that the crossing of the species barrier in humans may not emerge directly from the most frequent reservoir (i.e. white-tailed deer), as occurred with BSE (Bunk, 2004). For example,
CWD from cervids has showed amplified virulence and adaptation after spillover to rodents (Raymond et al., 2007), including rodent species overlapping with CWD-infected cervids in the wild (Heisey et al., 2010). Thus, even when the CDC states that 'If CWD could spread to people, it would most likely be through eating of infected deer and elk' (CDC, 2018a, https://www.cdc.gov/prions/cwd/prevention.html), disease ecology theory suggest that full assessments of zoonotic CWD risk should consider spillover from other species.

IV. ECOLOGICAL MODELLING OF CWD SPREAD, ZOONOTIC POTENTIAL, AND SPILLOVER

Infectious diseases are not distributed randomly across landscapes (Peterson, 2014; Escobar & Craft, 2016). Models accounting for landscape or climate configuration to quantify environmental conditions where spread of diseases occurs are used to understand distributional disequilibrium in the spread of diseases (Benavides, Valderrama & Streicker, 2016; Hutter et al., 2016; Piaggio et al., 2017), like CWD, that are undergoing range expansions. Interestingly, the available studies conducted at landscape levels also suggest that CWD does not occur randomly across taxonomic, temporal, geographic, and environmental spaces (Mathiason et al., 2009) (Fig. 4).

CWD was first linked to captive deer in Colorado (Fig. 1) (Williams & Young, 1980), but its subsequent spread has remained a mystery. Some detections have been linked to the translocation of infected captive cervids to previously uninfected cervid farms (Joly et al., 2003), including the spread of CWD in the USA and Canada (Evans, Schuler & Walter, 2014) and between Canada and Asia (Lee et al., 2013). For example, the transfer of infected captive cervids from the USA to Canada resulted in the spread of the CWD to at least one facility in Ontario and a captive cervid farm in Saskatchewan (Bollinger et al., 2004). Some detections of CWD in free-ranging cervids were preceded by detection in proximate captive herds, but this has not been consistent over the 40+ years of CWD spread (Olszowy et al., 2014; Haley & Hoover, 2015). In North America, over 175 captive cervid facilities have diagnosed CWD on their premises, with up to 80% of the herd infected in some cases (Carlson et al., 2018) (Fig. 2).

Spread of CWD in captive cervids suggests that transmission may be more effective in high-density herds and that facilities may act as effective point sources for infection (Bartelt-Hunt & Bartz, 2013; Zabel & Ortega, 2017). In North America, the spatial distribution of infected deer farms seems to follow a latent spatial process that appears clustered (Fig. 1). Similar patterns are observed in modelling wild populations (Joly et al., 2006), as disease prevalence typically declines with distance from heavily affected areas and landscape connectivity plays a larger role in the spread of disease (Conner & Miller, 2004; Joly et al., 2006; Williams & Young, 1980; Nobert et al., 2016). It is evident that CWD does not occur randomly in the geography, and geomorphology seems to play a role shaping its distribution (Fig. 5). However, coarse-scale biogeographic assessments of CWD distribution have not yet been performed. Similarly, the role of sampling bias in the structure of disease spread has not been studied in detail.

Sampling bias may limit our understanding of current CWD distribution (Conner, McCarty & Miller, 2000), with
the bulk of samples provided by hunters—i.e. current patterns of CWD distribution are influenced by sampling effort. A potential strategy to mitigate sampling bias would require rigorous analyses of environmental drivers underlying CWD dynamics in wildlife at different spatial and temporal scales (Plowright et al., 2008). Novel applications of multiscale modelling methods and theory from ecology and biogeography have facilitated identification of factors related to transmission, spread, and establishment of infectious diseases across large areas and a large number of species (Estrada-Peña et al., 2014; Peterson, 2014; Gortázar et al., 2014), but such approaches have never been applied to prion diseases, with most prion research conducted at population level (Fig. 4).

Beyond elk, deer (red, mule, white-tailed, black-tailed), and moose populations, the role of other species in CWD maintenance has not been explored extensively. Experimental data, generally using intra-cranial inoculation (Hamir et al., 2008), reveal that rodents (voles, mice, hamsters) (Bartz et al., 1998; Raymond et al., 2001; Heisey et al., 2010; Watts et al., 2014; Orrú et al., 2015), mesocarnivores (ferrets, mink, cats) (Bartz et al., 1998; Sigurdson et al., 2008; Perrott et al., 2013), livestock (cattle, sheep, pigs) (Hamir et al., 2001, 2005, 2006, 2007; Madsen-Bouterse et al., 2016; Moore et al., 2017), and other deer species (Reeve’s muntjac, Muntiacus reevesi and fallow deer, Dama dama) (Hamir et al., 2011; Nalls et al., 2013) are susceptible to infectious CWD prions. In *vivo* and *in vitro* models have produced mixed results regarding the ability of CWD to cross the species barrier into humans and livestock. To date, CWD remains restricted to cervids (Carlson et al., 2018), however, experimental work has identified a non-negligible spillover potential of CWD into humans or livestock (Mathiason et al., 2006; Haley et al., 2011). Uncertainty in the zoonotic potential of CWD, the magnitude of exposure of non-cervids to CWD, and a lack of tools to prevent CWD spread suggests that CWD is the prion disease with highest epidemiological risk (Jakob-Hoff et al., 2014).

To date, cervids are the only wildlife species monitored epidemiologically in routine CWD surveillance. Specific traits can help to evaluate the roles of other wildlife species as potential disease reservoirs (Luis et al., 2015). Recent studies showed that diverse ecological and evolutionary features describing intrinsic organismal characteristics can be combined to predict species suitable for disease transmission (Olive et al., 2017). For example, based on traits of species, recent studies used supervised machine learning algorithms to identify and prioritize rodent reservoirs of zoonotic diseases (Han et al., 2015), bat species hosting filoviruses (Han et al., 2016), mosquito vectors of Zika virus (Evans et al., 2017), and suitable tick vectors from the genus *Ixodes* (Yang & Han, 2018). Future research should explore species traits (e.g. phylogeny, physiology, behaviour) as predictors of associations between features of potential reservoir species, which will help to identify species that can be used in future experimental research, as sentinels for surveillance, and as animal models (Bancroft et al., 2011) (Fig. 2).

**V. ROLE OF THE ENVIRONMENT AND WILDLIFE IN THE SPREAD OF CWD**

Experimental studies have shown that infectious prions can enter the environment through saliva, faeces, urine, blood, antler velvet, or placenta tissue from infected animals, and carcasses (Angers et al., 2009; Zabel & Ortega, 2017). Importantly, CWD contamination of the environment *via* prion shedding in cervid excreta occurs many months before the onset of clinical disease (Mathiason et al., 2009; Plummer et al., 2017). Prions are hardy in the environment, are resistant to most general disinfectants (e.g. heating, most disinfectant chemicals, ultraviolet and ionizing radiation), and can remain infective for years to decades (Georgsson, Sigurðarson & Brown, 2006; Seidel et al., 2007; Smith, Booth & Pedersen, 2011). We recently reported that plants efficiently bind, uptake, retain, and transport infectious prions (Pritzkow et al., 2015). Other natural or man-made components of the environment, such as soil, rocks, wood, metals, and plastic, bind prions and do not diminish infectivity to susceptible species (Pritzkow et al., 2018). While oral ingestion
PrPSc in ecologically relevant environments, such as natural (Creech, e.g., in Wyoming) increase the risk of disease transmission (Thompson, Samuel & Van Deelen, 2008). For example, prolonging exposure to potentially contaminated areas increase disease transmission by exacerbating deer densities, (Fig. 2). Additionally, supplemental wildlife feeding can has been no detection of CWD in any of these species (Rees, 2014). Early warning systems, and guide hunting or culling to reduce surveillance, calculate sampling effort required to inform determination of CWD prevalence, identify locations for biogeographic-level studies will enhance our understanding of its occurrence across species, areas, and time periods (Levin, 1992). Available epidemiological data can be used to determine CWD prevalence, identify locations for surveillance, calculate sampling effort required to inform early warning systems, and guide hunting or culling to reduce CWD transmission (Rees et al., 2012). Epidemiological data can identify the location, species, and diagnostic method most effective for CWD surveillance and early detection, and to identify areas where citizen education and extension are crucial (Sorensen et al., 2014). International efforts are necessary for the development of standardized and systematic surveillance efforts in wildlife as an early warning system to anticipate CWD spread and the emergence of new prion diseases (Johnson, 2005).

Prion protein polymorphisms and strain diversity likely have important effects on the efficiency of prion transmission, so further knowledge of these aspects may contribute to the implementation of strategies for CWD reduction. Some reports have demonstrated the existence of distinct CWD prion strains (Angers et al., 2010; Crowell et al., 2015; Duque-Velásquez et al., 2015; Bian et al., 2019). However, limited tools exist to determine the origins and full diversity of natural prion strains in the wild—capitive interface of CWD (Igel-Egalon et al., 2018), limiting our capacity to identify the directions and effects of CWD spillover.

Epidemiological surveillance data coupled with landscape and community ecology analyses can help to determine how changes in the landscape and population configuration impact CWD circulation. For example, focal and consistent culling has been shown to reduce the prevalence of CWD in some wild cervid populations (Manjerovic et al., 2014; Sorensen et al., 2014), and simulations suggest that this approach can even eliminate the disease in certain situations (Potapov, Merrill & Lewis, 2012). This observation suggests that if CWD is discovered in high-value (endangered or conservation priority) isolated populations, where culling is not feasible, field testing and immediate culling of CWD-positive individuals could be economically and logistically feasible (Wolfe, Miller & Williams, 2004; Plummer et al., 2018). Combining theoretical approaches with surveillance data shows that deer density has varying levels of influence on contact rates and mechanisms of transmission (Storm et al., 2013; Potapov et al., 2013; Jennelle et al., 2014). Still, despite past debate on the density dependence of CWD transmission, it appears consistent that culling of CWD-positive individuals and landscape heterogeneity affect CWD prevalence at the population level (Conner et al., 2008; Wassergberg et al., 2009; Habib et al., 2011). Population models suggest that CWD generates selective pressures on deer populations and shapes the genetic diversity of populations by selecting for PrP genotypes associated with slower progression to clinical symptoms and death (Robinson et al., 2012). Overall, the frequencies of PrP genotypes associated with slower time to CWD death are low; importantly, no PrP genotypes are known that are truly resistant to CWD infection (Robinson et al., 2012). This selective process, however, is slow because of the chronic nature of CWD mortality. Williams et al. (2014) modelled the outcomes of a selective process on elk at the scale of decades to 100 years. The role that PrP genotypes play in shaping the population trajectories of CWD-infected cervid herds will likely be modified by hunting, which generally targets individuals in the oldest age class, of a specific sex (i.e. males may be targeted for trophy management or females targeted for population control), and acts at a time scale an
order of magnitude faster than PrP genotype selection (i.e. harvest causes non-selective mortality yearly versus selection occurring over decades or longer with regards to CWD genotype).

Considering the capacity of infectious prions to remain infective in specific landscape components (Fig. 2), identifying landscape configurations that facilitate CWD transmission is a high priority for future research. For example, analyses including CWD occurrence and specific vegetation phenologies, soil structure and composition, and local temperature and moisture, will allow researchers to identify landscape-level hotspots of CWD transmission risk to target deer control or landscape management. Thus, disentangling the landscape components that facilitate CWD transmission will expand the tools available to managers to modify such components via prescribed fire, habitat restoration, soil management, etc. to reduce their role as environmental reservoirs of CWD.

Beyond the landscape, other species in the community can influence CWD transmission. Empirical evidence supports the role of predators in the removal of sick and infectious prey across diverse disease systems (Packer et al., 2003). For example, grey wolf (Canis lupus) presence significantly reduced seroprevalence of bovine-virus-diarrhea in elk (Barber-Meyer & White, 2005), and mountain lions (Puma concolor) selectively predate on CWD-infected mule deer (Krumm et al., 2010). Other native large predators, such as grey wolves and bears (Ursus spp.), may similarly influence the prevalence and geographic distribution of CWD in wild reservoirs, as demonstrated through modelling applications (Hobbs, 2006; Wild et al., 2011). Additionally, numbers and geographic range of predators, including wolves and black bears (Ursus americanus), can be successfully managed and controlled via wildlife management methods (Meagher & Phillips, 1980; Clark, Huber & Servheen, 2002; Soorae, 2013).

Research assessing the role of predators in CWD transmission requires a multidisciplinary approach integrating expertise in human dimension, epidemiology, and ecology. Alternatively, carnivores and scavengers could potentially facilitate CWD spread to distant areas by translocating infectious prions from prey. This has been suggested for scats of coyotes (Canis latrans), raccoons (Procyon lotor) (Hamir et al., 2007; Moore et al., 2019), and crows (Corvus spp.) (Fischer et al., 2013), but has not been tested empirically. Scats may also be of potential utility in CWD surveillance and early detection, as predators can selectively predate CWD-infected cervids (Nichols et al., 2015). Whether predators can significantly improve the control and surveillance of CWD is unknown but deserves deeper exploration. Predator or scavenger scats have not been used in CWD surveillance to date.

**VII. CWD DETECTION**

Development of new diagnostic methods for disease detection can change interpretations of past research findings. In CWD research, methods used for the detection of prion-infected animals include immunohistochemistry (IHC) (Peters et al., 2000), enzyme-linked immunosorbent assay (ELISA) (Hibler et al., 2003), western blotting (WB) (Guiroy et al., 1993), protein misfolding cyclic amplification (PMCA) (Saborio, Permanne & Soto, 2001), and real-time quaking induced-conversion (RT-QuIC) (Henderson et al., 2015). All these methodologies are based on the detection of infectious PrPSc, but they have very different degrees of sensitivity and specificity. This disparity can lead to potentially inaccurate heuristics in detection procedures, and in turn, in our overall comprehension of CWD prevalence, distribution, and natural transmission.

Western Blotting, ELISA, and IHC detect PrPSc directly using specific antibodies. These techniques have been regarded as the ‘gold standard’ of official post-mortem diagnostic methods (Haley & Richt, 2017; USDA, 2019). Western Blotting, ELISA, and IHC, however, fail to identify low levels of PrPSc, which are likely present in animals recently exposed to CWD. On the other hand, PMCA and RT-QuIC show higher (ultra) sensitivity of detection than IHC, ELISA, or WB methods (Haley et al., 2009; Holcomb, Galloway & Mathiason, 2016). PMCA and RT-QuIC rely on the amplification of PrPSc using the same principle by which prions propagate during the disease. Both take advantage of the capacity of PrPSc to seed the conversion of PrPC into the abnormal form and employ a mechanical force to fragment the PrPSc aggregates, leading to the cyclic amplification of the prion replication process. These procedures enable specific detection of very small quantities of PrPSc in tissues and biological fluids, likely approaching the levels of single particles of PrPSc. Both PMCA and RT-QuIC have been used to detect CWD prions at high sensitivity and specificity in various tissues, fluids, and excreta (Pritzkow, Morales & Soto, 2014; Cheng et al., 2016; Krumm et al., 2017). Moreover, both PMCA (Saborno et al., 2001) and RT-QuIC (Orrú et al., 2017) have been reproduced extensively by many investigators around the world, and these technologies are currently being used in the diagnosis of human prion diseases in the USA and Europe. PMCA was developed first as a universal strategy for amplification of protein misfolding and RT-QuIC is basically a specific format of PMCA to carry out the process of amplification. In prion diseases, PMCA is normally done using brain homogenate as substrate for prion replication, using sonication as a mechanical force to break the aggregates in order to speed up the process, and traditionally utilizes WB for detection of the product. Conversely, RT-QuIC uses purified recombinant prion protein as a substrate, shaking as a fragmentation force, and fluorescence from an amyloid-binding dye as a readout. The main differences between PMCA and RT-QuIC in the context of prion replication is that PMCA reproduces better the biology of the disease, since the PrPSc generated after amplification is fully infectious and maintains the main features of prions, including strain diversity and the species barrier. On the contrary, RT-QuIC does not result in infectious material and does not reproduce strain features.
or the species barrier. Although a limitation in the study of prion biology, the lack of generation of infectivity by RT-QuIC might be an advantage for its application in routine detection, along with the fact that this assay is more practical for high-throughput screening.

A comparison of CWD detection methods found that all diagnostic methods (IHC, ELISA, WB, PMCA, and RT-QuIC) can successfully detect CWD infection post-mortem in advanced, terminal phases (McNulty et al., 2019). However, classic diagnostic methods, IHC, ELISA, and WB, failed to detect prion deposition at low concentrations, such as the expected amounts during the early phase of prion replication. By contrast, PMCA and RT-QuIC successfully detected prion presence even at very low concentrations, undetectable by traditional methods (McNulty et al., 2019). Thus, considering their insufficient sensitivity for diagnosis in acute phases, IHC, ELISA, and WB should not be used alone for early, asymptomatic CWD surveillance. Nevertheless, neither PMCA or RT-QuIC is yet employed in CWD surveillance, and both are still considered experimental (Gillin & Mawdsley, 2018). Thus, diagnostic uncertainty, direction of uncertainty (false-negative and false-positive rates), and consistent communication of the biological relevance of detection limits among diagnostic methods should be incorporated in reporting and analyses of CWD epidemiology.

CWD prions have been detected in several tissues of white-tailed deer (Fig. 6). However, tissue samples from the brain (obex) and medial retropharyngeal lymph nodes are the tissues most commonly used in IHC and WB analyses to detect the pathological accumulation of PrP Sc (Haley & Richt, 2017). Sampling these tissues, however, is highly invasive and requires post-mortem or expensive animal handling that limits the extent of samples available. New sampling strategies could be explored in routine surveillance programs, including ultrasensitive methods for prion detection and the use of less-invasive samples such as scat, saliva, or blood (Haley et al., 2011).

Recently, IHC detection of PrPSc in rectal biopsy was evaluated as ante-mortem test (Spraker et al., 2009; Thomsen et al., 2012; Monello et al., 2013). The diagnostic sensitivity of this assay was variable depending on the genotype of the animal and disease progression at the time of sample collection, ranging from 36 to 100% (Thomsen et al., 2012). Low sensitivity was observed in animals in the early stage of infection when the obex was negative for PrPSc and positive staining was only detected in medial retropharyngeal lymph nodes (Thomsen et al., 2012). Furthermore, although rectal
biopsy is relatively simple, it is still an invasive and expensive procedure.

Infectivity studies in deer or transgenic mice expressing the cervid prion protein have shown the presence of infectious materials in a large variety of tissues, including central nervous system tissues, peripheral nerves, lympho-reticular organs, gastro-intestinal tissues and skeletal muscle (Haley & Hoover, 2015). Infectivity was also found in various biological and excretory fluids, including blood, saliva, urine, and faeces (Haley et al., 2011; Kramm et al., 2017). However, it is likely that the quantity of PrP Sc present in these fluids is very small, orders of magnitude below the level of sensitivity of the commonly used ELISA and WB assays. Considering that PMCA can detect CWD in blood of infected cervids at the asymptomatic stage, this diagnostic method could be considered as an alternative for CWD detection and surveillance in biological and environmental samples (Kramm et al., 2017).

VIII. CONCLUSIONS

(1) Prions represent a unique type of wildlife pathogen that exhibit exceptional biological properties and large potential threats to wildlife conservation and human and animal health.

(2) Our understanding of transmissible spongiform encephalopathies (TSEs) has advanced dramatically because of CWD (Góñi et al., 2015). CWD has not been confirmed as a zoonotic disease, but research in this arena is still on-going.

(3) From an ecological perspective, control strategies could consider adopting new, ultrasensitive CWD detection procedures in biotic and abiotic reservoirs, management that confronts the interface of captive and wild cervids, restoration of natural predators of CWD reservoirs, and deer density and landscape modification to reduce CWD spread and prevalence.

(4) A more mature understanding of CWD detectability via modern ultrasensitive diagnostic methods would justify the cautionary use of previous epidemiological models based on data from low-sensitivity methods (e.g. ELISA).

(5) The elusive properties of prions have limited the study of their ecology in wild reservoirs, at least compared to other pathogens, and little is known regarding the predictability of prion disease spread among species and areas using classic methods in wildlife disease epidemiology and disease ecology.

(6) Multiscale ecological studies are necessary to untangle the ecological properties of prions at different temporal and geographic scales to understand their natural history in wildlife.

IX. ACKNOWLEDGMENTS

L.E.E. was supported by the Virginia Tech College of Natural Resources and the Environment Startup Funds and the Virginia Tech Destination Areas Rural Health Seed Grant #J0788219. C.S. was supported by NIH grant P01AI077774. We thank Manuel Jara for the cladogram of cervids and Sami Livingston for the drawings in Figure 2. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

X. CONFLICT OF INTEREST

C.S. is Founder, Chief Scientific Officer, and majority shareholder of AmpriIn Inc., a biotech company aiming to commercialize of PMCA and RT-QuIC technologies for highly sensitive detection of misfolded proteins implicated in various neurodegenerative diseases, including CWD. The University of Texas Health Science Center at Houston holds several patent applications related to the PMCA technology which have been licensed to AmpriIn Inc.

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