

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-Straussler-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, Brokx & 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

Table 1. Glossary of terms

Term	Definition
Amyloid	Protein aggregates with a characteristic intermolecular β -sheet-rich structure which can be stained with specific dyes, such as Congo red and Thioflavin S.
Bovine Spongiform Encephalopathy	BSE, commonly known as 'mad cow disease', is a prion disease of cattle. BSE is believed to have originated from scrapie, a prion disease of sheep. BSE generates variant Creutzfeldt–Jakob disease in humans exposed to infected beef.
Culling	Killing of targeted animals to reduce disease spread.
CWD	Chronic Wasting Disease is an infectious spongiform encephalopathy known to affect cervids, including mule deer, white-tailed deer, elk (or 'wapiti'), moose, and caribou.
ELISA	Enzyme-linked immunosorbent assay is a biochemical method used to detect antigens based on the use of specific antibodies to detect the presence of a target protein.
Feedgrounds	Artificial food subsidy generally used in wildlife management to sustain wild populations in periods of low natural food availability (e.g. supplementation of elk in Wyoming with square bales of grass or alfalfa during the winter).
Fibrils	Chains of protein aggregates adopting an amyloid structure generally associated with diseases.
Large carnivores	Large-sized predators such as mountain lion, bears, and wolves that have broad home ranges.
Mesocarnivore	Mid-sized predators such as a coyote, lynx, and raccoon that consume plants and fungi in addition to meat (~50% prey).
PMCA	Protein misfolding cyclic amplification is a diagnostic method based on reproducing the prion replication process in the laboratory in an accelerated manner through cycles of polymerization/fragmentation to multiply misfolded prions. A certain set of PMCA conditions is often referred as RT-QuIC.
Prions	Term coined by Stanley B. Prusiner after merging the words <i>proteinacious infectious agents</i> . It was originally used to denote the infectious agent responsible for prion diseases but recently has been used as a more general term to refer to any misfolded protein that can spread by seeding the conversion of normal proteins.
PrP	General abbreviation for prion protein.
PrP ^C	Normal PrP found in a healthy host.
PrP ^{Sc}	Infectious prion causing the spongiform encephalopathy scrapie in sheep, also used to denote the misfolded and infectious prion protein in general.
Scrapie	Neurodegenerative disease of sheep and goats caused by the infectious prion PrP ^{Sc} .
Spillover	Transmission of a pathogen between two different species.
Variant Creutzfeldt-Jakob Disease	Spongiform encephalopathy of humans caused by exposure to BSE.

where the PrP sequence difference is rather small, the species barrier is manifested as a prolongation of the time it takes for animals to develop the clinical disease when inoculated with another species' infectious material (Robinson *et al.*, 2012). Transmission of prion disease has been shown between animals and humans, with a notorious outbreak caused by the transmission of Bovine Spongiform Encephalopathy (BSE) to produce variant Creutzfeldt-Jakob Disease in humans (Ironsides *et al.*, 1996).

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; Evisa, 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

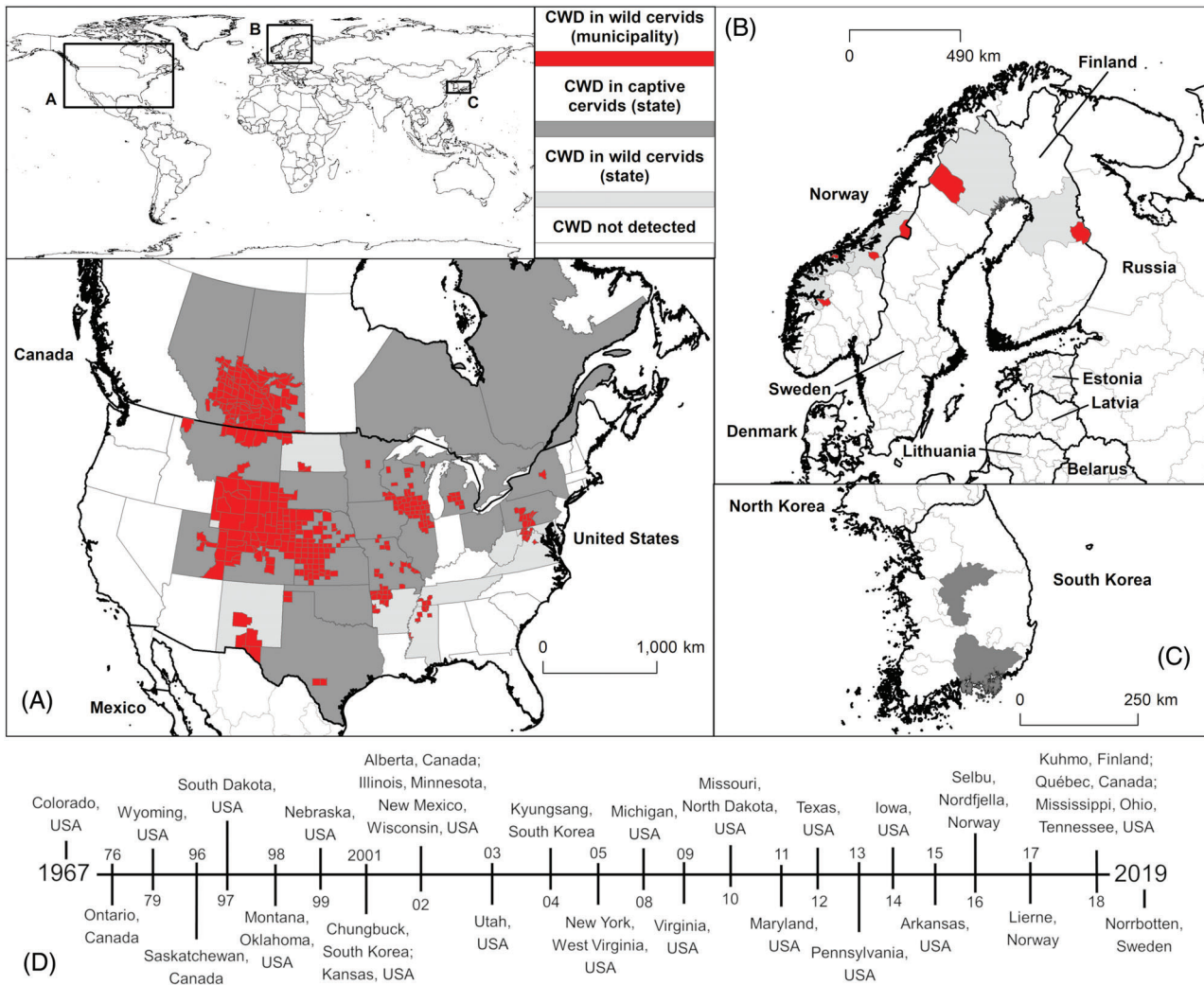


Fig. 1. Geographic distribution of Chronic Wasting Disease (CWD) reports. (A) The region with the most cases and areas infected with CWD is North America; (B) Europe has reported CWD in Norway, Sweden, and Finland; (C) Asia reported CWD in South Korea. Red: counties (in USA) and wildlife management areas (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, 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; Vikøren *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; Carlson *et al.*, 2018). The highest infection rate has usually been found in older males followed by

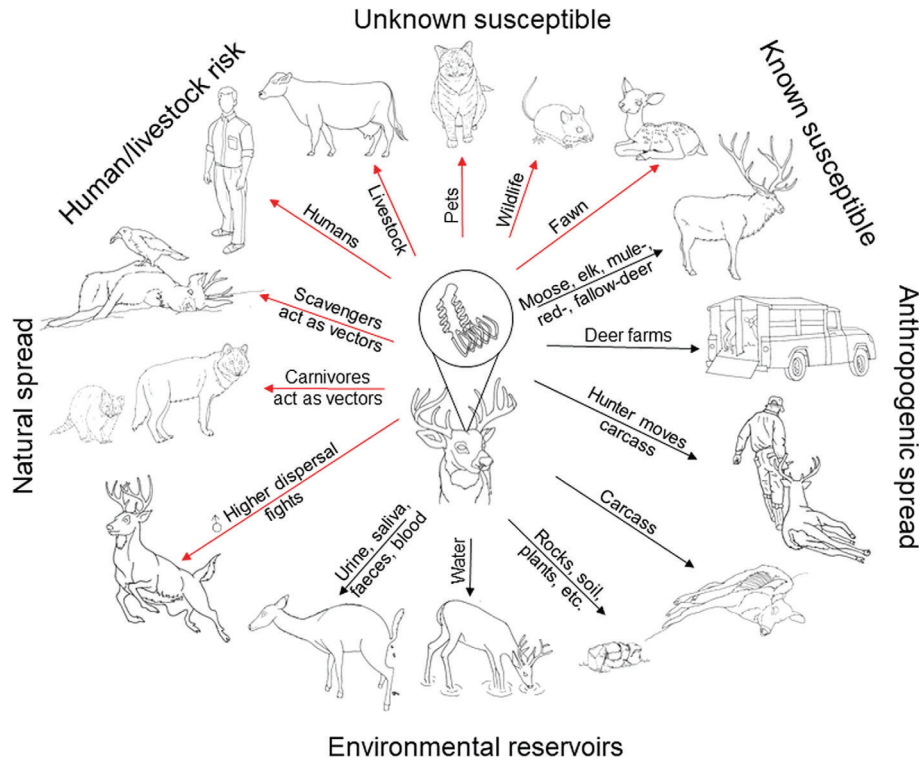


Fig. 2. Role for intermediate species and environmental reservoirs in the spread of Chronic Wasting Disease (CWD). Wild cervid populations serve as the reservoirs of CWD (Carlson *et al.*, 2018), acting as source for spillover to other species and the environment. Natural spread: spread in natural areas 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 PrP^{Sc} 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 of infected deer). 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

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, ZONOTIC 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;

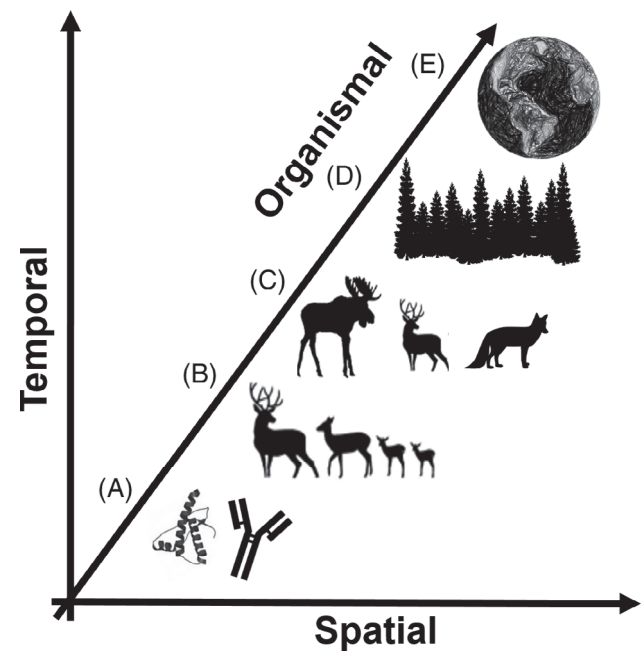


Fig. 4. Different dimensions and scales (micro to macro) to study Chronic Wasting Disease (CWD). Three dimensions could be used to study CWD: spatial, temporal, and organizational dimensions. The x axis denotes the spatial dimension (from millimetres to continents); the y axis denotes the temporal dimension (from seconds to centuries); the z axis denotes the organizational dimension (from molecules to biomes). Micro: (A) molecular-level studies focus on prion detection and characterization, e.g. *in vitro* measurements on prion conversion; (B) population-level studies explore transmission between individuals of a group, e.g. transmission experiments in captive deer; and (C) community-level studies explore potential CWD spillover among species, e.g. evaluating the role of other wild species in prion dissemination and maintenance. Medium: (D) landscape-level studies aim to identify biotic and abiotic factors associated with CWD maintenance and spread in endemic areas, e.g. assessing CWD distribution based on landscape configuration among seasons. Macro: (E) biogeographic-level studies aim to understand factors associated with CWD spread among large regions, periods, and communities, e.g. exploring climatic drivers of CWD occurrence at a continental level or long-distance spread from translocation of captive cervids. The different organizational levels can be studied across different geographic and temporal scales (e.g. from fine to coarse). Most CWD research has been restricted to studies at low organization level and fine temporal and spatial scales. Studies at the community, landscape, and biogeographic levels underlying CWD occurrence remain neglected and are critically needed.

Blanchong *et al.*, 2008; 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

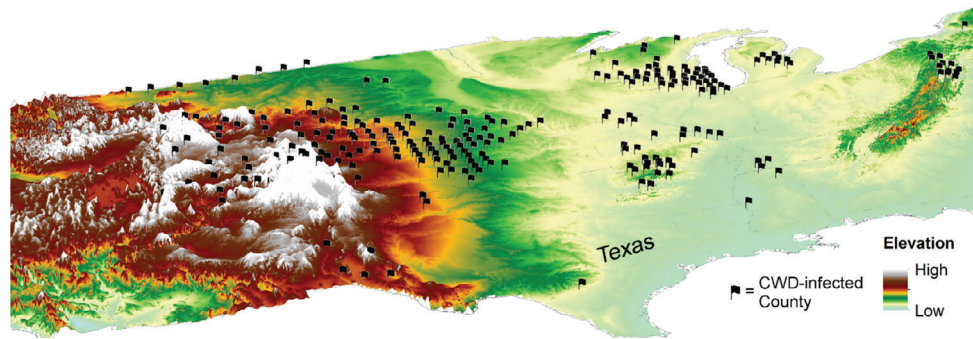


Fig. 5. Geomorphology and Chronic Wasting Disease (CWD). USA counties reported as CWD-positive in wildlife (black flags). Note the geomorphology of the country with areas of low (blue) and high elevation (white). The map suggests that CWD is broadly found in flat landscapes with plausible interruption or retardation of spread in the highlands of western (e.g. Utah, Idaho, western Colorado) and eastern regions (e.g. Appalachian Mountains).

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.*, 2007; 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 vitro* and *in vivo* 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 (Olival *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, Sigurdarson & 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

is the most common route of exposure for prion disease, infectious scrapie prions caused 100% mortality *via* airborne transmission in a laboratory challenge of mice (Haybaeck *et al.*, 2011). One report suggested that prions bound to soil are more infective than free prions, so soil may serve both as an environmental reservoir and a facilitator of CWD prion transmission (Johnson *et al.*, 2007). Soils of different texture, mineralogy, and organic content appear to bind differently to prions and show distinct infectivity rates *via* oral or aerosolized transmission (Johnson *et al.*, 2007). For example, soils with high organic material content (e.g. high concentration of humic acid) appear to degrade CWD prions faster (Kuznetsova *et al.*, 2018).

Direct exposure of non-cervid animals to CWD-infected cervids could help to spread CWD. For example, hawks, owls, crows, dogs, cats, coyotes, raccoons, skunks, mink, foxes, and opossums that consume deer carcasses could act as spillover hosts and potentially vector spreaders of CWD (Bunk, 2004; Jennelle *et al.*, 2009), although there has been no detection of CWD in any of these species (Fig. 2). Additionally, supplemental wildlife feeding can increase disease transmission by exacerbating deer densities, increasing contact rates, altering normal behaviour, and prolonging exposure to potentially contaminated areas (Thompson, Samuel & Van Deelen, 2008). For example, feeding grounds used to subsidize wild elk during the winter (e.g. in Wyoming) increase the risk of disease transmission (Creech *et al.*, 2012). Similarly, recent detection of CWD PrP^{Sc} in ecologically relevant environments, such as natural mineral licks where wildlife obtain minerals from soil and water consumption, suggests a key role of landscape features in CWD transmission risk (Plummer *et al.*, 2018). Thus, identifying where and when CWD occurs in a timely fashion can help inform policies regarding baiting and supplemental feeding of cervids and to consider direct habitat modification with the aim of reducing infectious contact (Sorensen, van Beest & Brook, 2014). Identifying the environmental conditions that facilitate or limit CWD infectivity, as well as the relative importance of CWD transmission from environmental reservoirs *versus* direct animal–animal transmission, may help to identify potential control methods (Gear *et al.*, 2010).

VI. STRATEGIES TO CONTROL THE SPREAD OF CWD

Expanding CWD research from fine-scale to landscape- and 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–captive 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; Wasserberg *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 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 PrP^{Sc}, 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 PrP^{Sc} 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 PrP^{Sc}, 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 PrP^{Sc} using the same principle by which prions propagate during the disease. Both take advantage of the capacity of PrP^{Sc} to seed the conversion of PrP^C into the abnormal form and employ a mechanical force to fragment the PrP^{Sc} aggregates, leading to the cyclic amplification of the prion replication process. These procedures enable specific detection of very small quantities of PrP^{Sc} in tissues and biological fluids, likely approaching the levels of single particles of PrP^{Sc}. 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; Kramm *et al.*, 2017). Moreover, both PMCA (Saborio *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 PrP^{Sc} 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

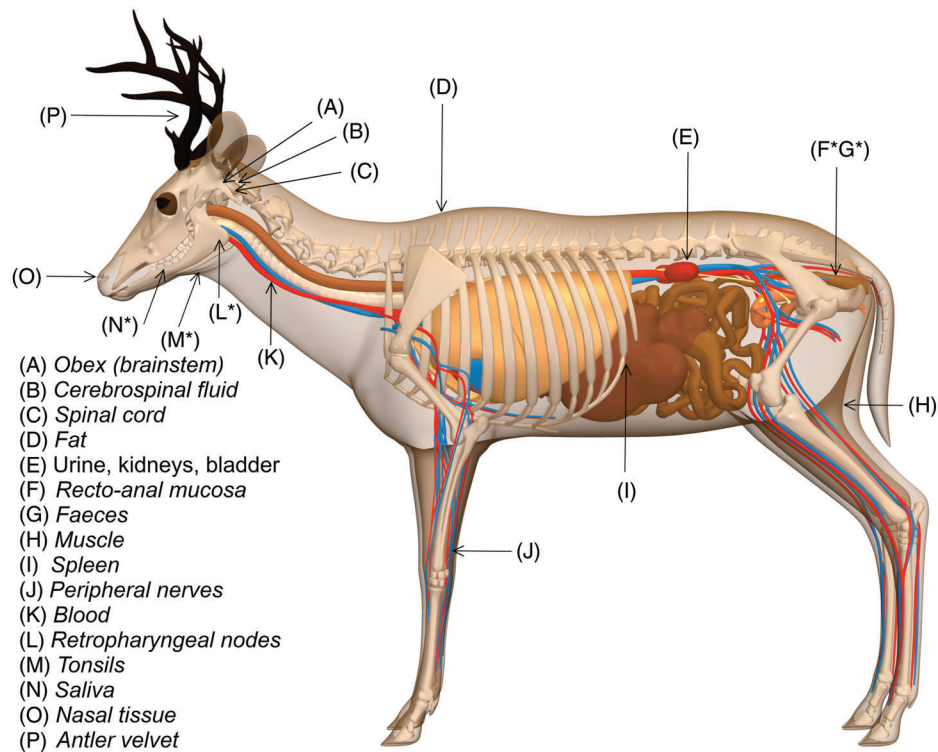


Fig. 6. Detection of Chronic Wasting Disease (CWD) in cervid tissues. Distribution of deer tissues used in CWD detection. (A) obex and brainstem (Haley *et al.*, 2011); (B) cerebrospinal fluid (Haley & Hoover, 2015); (C) spinal cord (Baeten *et al.*, 2007); (D) fat (Race *et al.*, 2009); (E) urine, kidneys, bladder (Haley & Hoover, 2015); (F) lymphoid tissue in the intestines including intestinal and rectoanal mucosa (Haley *et al.*, 2011); (G) faeces (Tamgüney *et al.*, 2009); (H) muscle (Angers *et al.*, 2009); (I) spleen (Sigurdson *et al.*, 2002); (J) peripheral nerves (Seelig *et al.*, 2011); (K) blood (Mathiason *et al.*, 2006); (L) medial retropharyngeal lymph nodes (Sigurdson *et al.*, 2002); (M) tonsils (Sigurdson *et al.*, 2002); (N) saliva (Mathiason *et al.*, 2006); (O) nasal tissues (Haley *et al.*, 2016); (P) antler velvet (Angers *et al.*, 2009). Asterisks indicate whether sensitivity is >50% based on Haley & Richt (2017).

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 used of less-invasive samples such as scat, saliva, or blood (Haley *et al.*, 2011).

Recently, IHC detection of PrP^{Sc} 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 PrP^{Sc} 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 (Goñi *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.

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X. CONFLICT OF INTEREST

C.S. is Founder, Chief Scientific Officer, and majority shareholder of Amprion 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 Amprion Inc.

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