Chronic Wasting Disease: Current Assessment of Transmissibility

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Abstract

Chronic wasting disease (CWD) is a prion disease of cervids characterized by clinical symptoms of progressive weight loss, abnormal behaviour and excessive salivation. Incidents have been reported in North America and Korea as well as in Europe. Current knowledge, based on *in vitro* and *in vivo* experiments, suggests direct CWD transmission to humans is unlikely. Nonetheless, humans may consume CWD-infected materials, which presents a potential risk. Studies indicate that transmission by horizontal infection of cervids probably occurs via saliva, faeces, and urine as well as from environmental reservoirs of prions found in soil and water. In addition, infectivity in the skeletal muscle of infected deer has been observed. These findings suggest that direct contact with infected animals and indirect contact with prion-contaminated materials are potential sources of infection. However, recent studies on the detection of pregnancy-related prion infectivity imply the potential transmission of CWD from mother to offspring. In this review, fundamental aspects of CWD are reviewed.

History of chronic wasting disease (CWD) and its current status

Chronic wasting disease (CWD) is a fatal neurodegenerative prion disease that affects cervids. CWD is transmitted between a range of cervid species such as mule deer (*Odocoileus hemionus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), red deer (*Cervus elaphus elaphus*), elk (*Cervus Canadensis*), reindeer (*Rangifer tarandus*), and Shira's moose (*Alces alces shirasi*), Reeves' muntjac deer (*Muntiacus reevesi*) (Williams *et al.*, 1980, 1982; Baeten *et al.*, 2007; Nalls *et al.*, 2013; Haley *et al.*, 2015; Benestad *et al.*, 2016).

The first report of CWD described a wasting disorder in mule deer caught for nutrition

research near Fort Collins, Colorado in 1967 (Williams *et al.*, 1980). In 1978, pathologists Elizabeth Williams and Stewart Young established that CWD is a form of spongiform encephalopathy (Williams *et al.*, 1980). It was subsequently discovered that CWD not only results in typical vacuolation in neurons (Williams *et al.*, 1980) but also prion protein (PrP) accumulation (Spraker *et al.*, 2002) and infectivity in the brain (Browning *et al.*, 2004).

In 1981, a captive CWD-infected elk was found in Colorado, and then in 1985 a captive CWD-infected mule deer was also identified. Increased surveillance led to the discovery of CWD in Wyoming. To date, the disease has been reported in 24 American states, two Canadian provinces, and South Korea (National Wildlife Health Center, 2018).

The first case of CWD to be detected in Europe (Norway) was in wild Norwegian reindeer (Benestad *et al.*, 2016). In March 2018, Finland also recorded a case of CWD in a wild moose (Finnish Broadcasting Company, 2018). The disease identified in reindeer from Europe is similar to that of CWD in North America. However, CWD of moose in Europe has some distinct features, suggesting a new CWD strain that is different from the CWD strain in North America (EFSA, 2017).

Susceptibility and environmental reservoirs of CWD

The prevalence of CWD is increasing and is currently present in > 30% of some free-ranging herds (Edmunds *et al.*, 2016) and more than 80% of captive herds (Keane *et al.*, 2008b).

The origins of the disease remain unclear. Saprovores, such as buzzard and jackal, and other animals, such as mountain lion, fox, raccoon, coyote and eagle, prey on deer and elk. Thus, CWD may potentially spread widely among a variety of animals. Apart from cervids, CWD-susceptible animals include ferret (Bartz *et al.*, 1998), raccoon (Hamir *et al.*, 2003), squirrel monkey (Marsh *et al.*, 2005), cattle (Hamir *et al.*, 2005, 2007), sheep (Williams, 2005; Hamir *et al.*, 2006), goat (Williams *et al.*, 1992), hamsters (Bartz *et al.*, 1998; Raymond *et al.*, 2007), bank voles (Heisey *et al.*, 2010; Di Bari *et al.*, 2013), mink (Harrington *et al.*, 2008), meadow voles (Heisey *et al.*, 2010), red backed voles (Heisey *et al.*, 2010), white-footed mice (Heisey *et al.*, 2010), deer mice (Heisey *et al.*, 2010) and cats (Mathiason *et al.*, 2013).

The stability of prions in the environment remains unclear at the present time. For example, the concentration of prions in seawater and rivers is unknown. It has been demonstrated that clay components of soil bind to CWD prions, which may contribute to the environmental stability of the bound prion particles (Wyckoff *et al.*, 2016). Indeed, naturally contaminated soil was shown to contain infectious CWD prions that could be experimentally transmitted to elk PrP-expressing transgenic mice (Wyckoff *et al.*, 2016). Surprisingly, soil-bound prions have enhanced infectivity via the oral route compared to unbound prions (Johnson *et al.*, 2007). In addition, using serial protein misfolding cyclic amplification (PMCA), very low levels of proteinase K (PK)-resistant cervid PrP (i.e. an abnormal isoform of cervid PrP, PrP^{CWD}) were detected in environmental water (Nichols *et al.*, 2009) and mineral licks (Plummer *et al.*, 2018) from a CWD endemic area. Moreover, a recent study reported that CWD prions interact with vegetation of prairie and boreal regions (Kuznetsova *et al.*, 2018). These findings suggest that environmental materials, such as soil and water, may be an important reservoir of prion infectivity and a source of CWD infection. Taken together, these observations highlight the importance of minimizing environmental pollution of CWD prions, particularly in soil and water.

Clinical symptoms and pathogenesis of CWD

The characteristic clinical symptoms of CWD-infected animals are slow progressive neurological dysfunction, weight loss, behavioural changes, emaciation, excessive salivation, teeth grinding, fever, anorexia, polyposia, dysphagia, excessive urination, impaired motor coordination and respiratory distress (Sohn *et al.*, 2002; Williams, 2005; Haley *et al.*, 2015). Because these symptoms are not CWD-specific, additional biochemical and pathological analysis is required to verify the diagnosis. Although CWD-infected deer displaying symptoms of the disease can be found throughout the year, infected carcasses are most common in the winter period. The relative profusion of infected carcasses at this time of the year is probably due to the severe winter climate in North America. Furthermore, most deer displaying symptoms of CWD are three to four years of age.

After infection with CWD agent, cellular PrP (PrP^{C}) is converted into PrP^{CWD}, which accumulates in brain tissue causing neurological symptoms and ultimately death. PrP^{CWD} is primarily distributed in the central nervous system (CNS) and lymphatic tissues, including brain, spinal cord, tonsil, lymph node and spleen. Small quantities of PrP^{CWD} agent are also found in the heart and eyes of infected deer. In addition, deboned muscle (Angers *et al.*, 2006) and fat (Race *et al.*, 2009) as well as antler velvet (Angers *et al.*, 2009) have proven to be infectious. Prion infectivity was found in the pregnancy microenvironment of cervids, including the uterus, placenta, ovary, and the placentome (Nalls *et al.*, 2017, 2018). Importantly, CWD prions have been detected in body fluids and excreta such as saliva (Haley *et al.*, 2009a), urine (Haley *et al.*, 2009a), faeces (Tamgüney *et al.*, 2009), amniotic fluid (Nalls *et al.*, 2017), cerebrospinal fluid (Nichols *et al.*, 2012) and blood (Mathiason *et al.*, 2006) (Table 9.1), which may be an important source of infection.

Orally infected CWD-brain homogenate of mule deer induces PrP^{CWD} accumulation as early as 42 days post infection in intestine-associated lymphatic tissues, such as retropharyngeal lymph nodes, tonsil, Peyer's patch and ileocecal lymph nodes. Therefore, it has been proposed that CWD agent may be transmitted via clinical and pre-clinical animal carcasses as well as from by-products of deer, such as saliva, stools and urine (Miller *et al.*, 2004; Mathiason *et al.*, 2006; Haley *et al.*, 2009a; Tamgüney *et al.*, 2009). More importantly,

Table 9.1 CWD prions in body fluids and excreta

Sample type	Species	References
Saliva	White-tailed deer	Haley *et al.* (2009a), Mathiason *et al.* (2009)
Saliva	Mule deer	Mathiason *et al.* (2006, 2009), Tamgüney *et al.* (2012)
Urine	White-tailed deer	Haley *et al.* (2009a,b)
Blood	Mule deer	Mathiason *et al.* (2006)
Blood	White-tailed deer	Mathiason *et al.* (2009)
Amniotic fluid	Muntjac deer	Nalls *et al.* (2017)
Cerebrospinal fluid	Elk	Nichols *et al.* (2012)
Cerebrospinal fluid	White-tailed deer	Haley *et al.* (2013)
Faeces	Mule deer	Tamgüney *et al.* (2009)
Faeces	White-tailed deer	Haley *et al.* (2009b)

skeletal muscle of CWD-infected deer causes CWD in cervid PrP expressing transgenic mice (Angers *et al.*, 2006). This finding indicates that muscle is a potential source of infection, suggesting that the meat of deer must be treated with caution.

At this time it remains unclear how CWD is spread. Natural or anthropogenic movement of animals may play an important role in the dissemination of CWD. Direct contact between animals and indirect transmission *via* soil and body surfaces are potential mechanisms for the spread of the disease. CWD is thought to be horizontally infected at a high rate among cervids (Miller *et al.*, 2003). Oral transmission of CWD-infected brain homogenate derived from mule deer to elk has been reported, which is further supported by the finding of CWD-infected free-ranging moose (Baeten *et al.*, 2007). Transfer of infectious material in saliva and/or stools is currently considered a likely means of transmission. The movement of animal carcasses also seems to be involved in the dispersion of CWD. This proposal is supported by evidence that an infected carcass placed in a field resulted in the transmission of CWD to experimental mule deer (Miller *et al.*, 2004), suggesting the spread of CWD may result from a contaminated CWD environment. Recent research has detected the peripheral accumulation of prions and the shedding of infectious prions in bodily fluids, which can be detected even before the appearance of clinical signs (Haley *et al.*, 2017). These findings suggest that not only clinical but also preclinical animals as well as carcasses and by-products may contribute to the transmission and spread of CWD. There is also evidence for the horizontal transmission of CWD based on epidemiological data as well as experimental model studies using transgenic mice expressing the normal cervid prion protein (Seelig *et al.*, 2010).

CWD strains

Currently, the number of CWD strains and their prevalence, as well as transmission properties, remain unclear. Recent studies indicate the presence of conformational variants or strains of CWD prions may exist. Indeed, sequential passage of a single mule deer CWD isolate using hamster or hamster-PrP expressing transgenic mice causes two distinct disease phenotypes (Raymond *et al.*, 2007; Heisey *et al.*, 2010). Moreover, LaFauci *et al.* (2006) have reported that elk PrP-expressing transgenic mice developed phenotypically divergent diseases when inoculated with either mule deer or elk CWD, which was suggestive of different strains among cervids. These studies, together with other bioassay data, suggest the presence of predominant strain types comprising at least two, and possibly three, distinct CWD strains in North America (e.g. CWD1 and CWD2; Sgha CWDmd-f and Sgha CWDmd-s; CWD-WI and CWD-CSU; WST and CKY) (Raymond *et al.*, 2007; Angers *et al.*, 2010; Bessen *et al.*, 2011; Perrott *et al.*, 2012; Crowell *et al.*, 2015; Triscott *et al.*, 2015; EFSA, 2017). However, the newly discovered CWD prion in Norway seems to be different from the above-mentioned CWD prion strains (Bian *et al.*, 2018).

Relationship between CWD and human prion diseases

Several animal prion diseases, such as scrapie, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy, feline spongiform encephalopathy, and ungulate spongiform encephalopathy, display similar symptoms to CWD. However, there is no known relationship between CWD and these animal prion diseases. In addition, epidemiological

studies showed no correlation between CWD and human prion diseases (Belay *et al.*, 2004; Anderson *et al.*, 2007; Oszowy *et al.*, 2014; Haley *et al.*, 2015). Most laboratory studies also suggest that the risk of CWD transmission to humans is low. This conclusion is supported by a study in which intracerebral inoculation of CWD prions to transgenic mice expressing human PrP did not lead to the development of any hallmark symptoms of CWD after > 650 days, while an equivalent inoculation to transgenic mice expressing cervid PrP resulted in infection within 150 days (Kong *et al.*, 2005). These findings indicate the presence of a barrier that makes interspecies transmission unlikely, especially between species as diverse as humans and deer (Kong *et al.*, 2005). Moreover, an *in vitro* assay showed PrP^{CWD} mediated conversion of human PrP to a protease-resistant form is very inefficient, suggesting a species barrier at the molecular level that limits the susceptibility of humans to CWD (Raymond *et al.*, 2000).

In marked contrast, transmission experiments involving non-human primates have shown mixed results. For example, squirrel monkeys were found to be susceptible to CWD infection (Race *et al.*, 2014), although there was no clinical, pathological, or biochemical evidence of CWD transmission to cynomolgus macaques (Race *et al.*, 2018). Currently, it is unclear why cynomolgus macaques and squirrel monkeys give markedly different results with regard to CWD transmission. Although the conclusions to be drawn from studies using non-human primates as infection models are still a matter of debate, a plethora of laboratory experiments suggest the risk of CWD transmission to humans is low (Hannaoui *et al.*, 2017). Nonetheless, the potential zoonotic transmission of CWD is an alarming prospect. Given that the scientific information on CWD is limited, consumption of CWD-positive animals should be prohibited at this time.

Diagnostic methods for CWD

The gold standard of CWD diagnosis is immunohistochemistry (IHC) (Haley *et al.*, 2017). Medullary obex and lymphatic tissues, such as tonsil and pharyngeal lymph nodes, are used for this analysis. Dorsal motor nucleus of the vagus (DMNV) in the obex region of the brainstem and the medial retropharyngeal lymph nodes (RLN) is generally used for regulatory diagnosis (Sigurdson *et al.*, 1999; Peters *et al.*, 2000; Keane *et al.*, 2008a). IHC is a method of observing tissue sections stained with anti-PrP antibody using light microscopy. This methodology has merits in terms of its high level of sensitivity and specificity. In addition, IHC can be used to verify whether the tissue distribution of PrP^{CWD} is as anticipated. Other methods for CWD diagnosis include enzyme-linked immunosorbent assay (ELISA) and western blotting. ELISA is used to detect PrP adsorbed on plates after treatment of brain homogenate with PK. Western blotting detects PrP absorbed on a membrane after an electroblotting procedure. ELISA is preferable because the technique allows rapid and quantitative analysis. However, western blotting facilitates more detailed biochemical analysis such as determination of the molecular weight of PrP. Such information may be used to help identify the prion source and specific strain.

The United States Department of Agriculture (USDA) has recently certified an ELISA kit as an official screening test for CWD using obex and lymph node tissues (USDA, 2017). Further developments in detecting CWD have enabled antemortem diagnosis using IHC of tonsil tissue. In addition, a method for prion diagnosis, known as PMCA, has been developed that amplifies PK-resistant PrP by sequential incubation and sonication using a mixture of

PrP^{C} source and PrP^{CWD} seed. PMCA can detect PrP^{CWD} in the urine of presymptomatic deer (Rubenstein *et al.*, 2011) and the blood of asymptomatic deer (Kramm *et al.*, 2017).

More recently, a real-time quaking-induced conversion (RT-QuIC) assay, which is a modified version of PMCA for enabling the quantification of PrP^{CWD} at high sensitivity, has been developed (Manne *et al.*, 2017). Using this methodology it is possible to perform antemortem detection of CWD in recto-anal mucosa-associated lymphoid tissue (RAMALT). The performance of RT-QuIC showed good results at 92% relative sensitivity and 95% relative specificity using blinded samples compared to IHC results. Other researchers also detected PrP^{CWD} from RAMALT and nasal brushings using RT-QuIC (Haley *et al.*, 2016).

Powerful research tools to analyse CWD will be invaluable in helping to reveal the molecular basis of the disease. For example, a transgenic mouse expressing cervid PrP (Browning *et al.*, 2004; Seelig *et al.*, 2010) and a CWD-susceptible cell line (Raymond *et al.*, 2006) have been developed. Such tools will be used to devise bioassays of CWD infectivity in tissues and body fluids derived from CWD-infected cervids.

Taken together, the recent development of diagnostic technologies, such as qualitative and quantitative amplification techniques, enables not only postmortem but also antemortem detection of PrP^{CWD} from peripheral tissues and body fluids. In addition, these diagnostic methods will contribute to further risk assessment of deer tissues and body fluids obtained from CWD-endemic areas as well as improving surveillance programs.

Conclusion and perspectives

There is a strikingly high incidence of CWD in wild animals, such as deer and elk, which maintains a reservoir of CWD prions that is difficult to counteract by the simple approach of targeted culling. In addition, the high stability of CWD prions in the environment further exacerbates the problem. Moreover, the diverse distribution of CWD prions within the body of infected cervids, including neurological and peripheral tissues as well as muscle, fat and pregnancy tissues, increases the chances of transmitting the disease. In particular, the presence of CWD prions in body fluids, such as saliva, faeces and urine, enhances infectivity and may lead to both horizontal and vertical transmission. Although more than two CWD strains have been found, their host range and prevalence as well as transmission features remain unclear at the present time.

To date, laboratory studies and epidemiological investigations suggest little association between human prion diseases and exposure to CWD prions. However, it is not known whether there is an absolute species barrier for humans against all strains of CWD prions. Thus, long-term investigations to study potential human infection and exposure to CWD prions are needed.

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