REVIEW

CHRONIC WASTING DISEASE

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Summary. – Chronic wasting disease (CWD) is the only known prion disease affecting free-ranging animals and has become a serious epidemic in North America. Although any case was reported from Europe, the spread of the disease to other continents and regions cannot be excluded, because the transmission of CWD is the most efficient among prion diseases. This article reviews the host range of CWD including experimentally infected animals, models for potential transmissibility to humans, clinical signs of the disease, pathogenesis, and methods for CWD detection.

Key words: chronic wasting disease; cervids; transmission; surveillance

Contents

1. Introduction

Transmissible spongiform encephalopathies (TSE) or prion diseases belong to the conformational neurodegenerative disorders (Sadowski and Wisniewski, 2007; Prusiner, 2001). This category includes CWD, a generally known prion disease of the free-ranging animals. This group of disorders is characterized by a conformational change of normally expressed prion protein (PrP) to the insoluble misfolded, ß-sheet rich conformer resistant to proteolytic degradation that is toxic to neurons (Fig. 1). Accumulation of the misfolded prion protein in the brain leads to a neurodegeneration and ultimately to death. Clinical

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Abbreviations: aa = amino acid; APHIS = Animal and Plant Health Inspection Service; BSE = bovine spongiform encephalopathy; CWD = chronic wasting disease; PrP = prion protein; PrP^{C} = cellular prion protein; PrP^{CWD} = CWD-associated prion protein; PrP^{Sc} = scrapie-associated prion protein; USDA = United States Department of Agriculture; TSE = transmissible spongiform encephalopathies

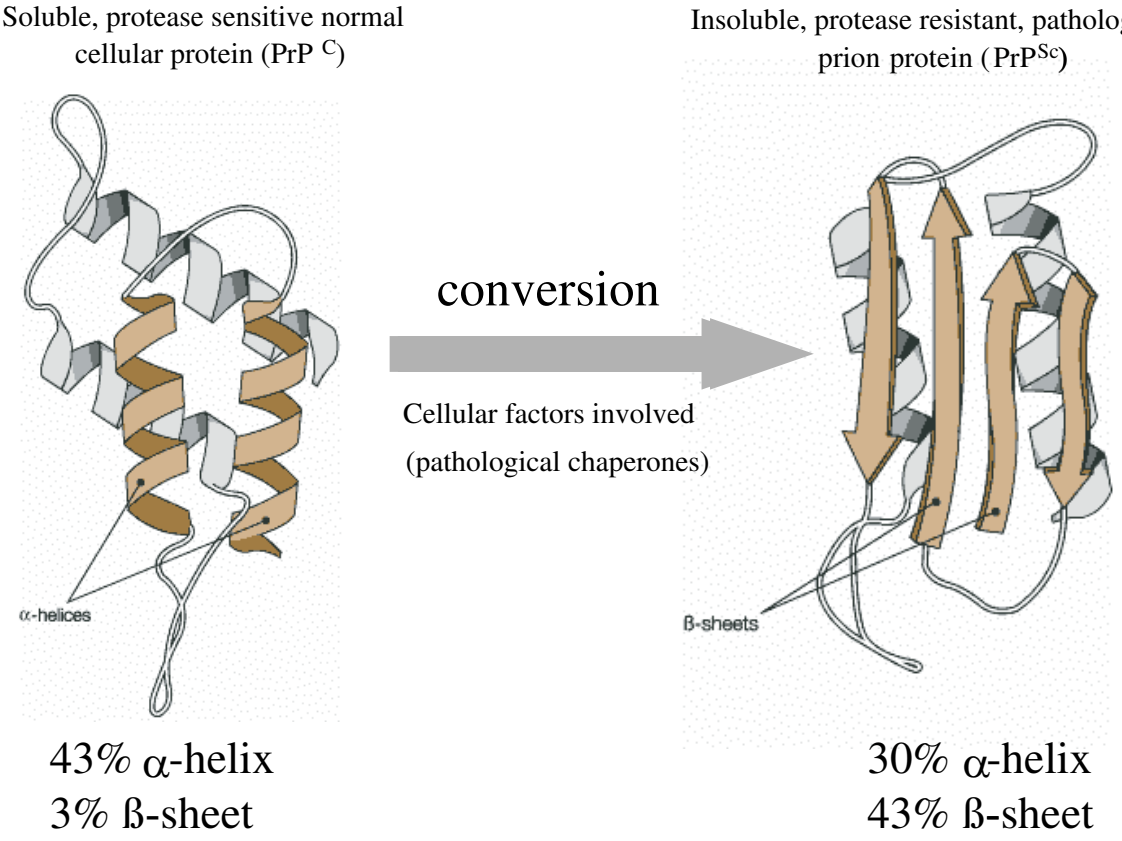

Fig. 1

Conversion of cellular PrP^{C} to pathological scrapie-associated PrP^{Sc}

Modified according to Prusiner (1999).

manifestations of CWD include weight loss, behavioral changes, salivation, severely affected swallowing, ataxia, and head tremors (Williams and Young, 1982; 1980).

Initially, CWD was characterized as a fatal wasting syndrome in a captive mule deer within a research facility in Fort Collins, USA, in 1967. At first, the disease was considered as associated with captivity followed by a nutritional deficiency. After more than a decade, it was recognized as TSE based on the presence of characteristic neuropathological lesions (Williams and Young, 1980). Later, cases of this disease in the farmed elk were discovered (Williams and Young, 1982). At the outset of the disease, CWD-positive free-ranging animals have been identified in elk, mule deer, and white-tailed deer populations (Williams and Miller, 2002). Most vulnerable to CWD infection is white-tailed deer and at present the disease is found in areas with the large population of these animals (Wisniewski and Sigurdsson, 2007). Recently, the first case of free-ranging moose affected with CWD was reported (Baeten *et al*., 2007). CWD is now endemic in Colorado, Wyoming, and Nebraska and spreads to other parts of USA (Wisniewski and Sigurdsson, 2007). The widespread occurrence of CWD in farmed and free-living animals led to the intensive CWD research focused on the understanding of epidemiology, susceptibility, transmission, pathogenesis, and PrP structure.

2. Host range

CWD affects members of the family *Cervidae,* namely mule deer (*Odocoileus hemionus*), including subspecies *O.h. hemionus* and *O.h. columbianus*, white-tailed deer (*O. virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces shirasi*) (Baeten *et al*., 2007; Spraker *et al*., 1997; Williams and Young, 1992). Reindeer (*Rangifer tarandus*) has a PrP sequence highly homologous with mule deer PrP, and therefore is likely susceptible to CWD (Sigurdson and Aguzzi, 2007). European cervids, moose and red deer (*Cervus elaphus elaphus*) are expected to be CWD-susceptible, too (Williams *et al*., 2002).

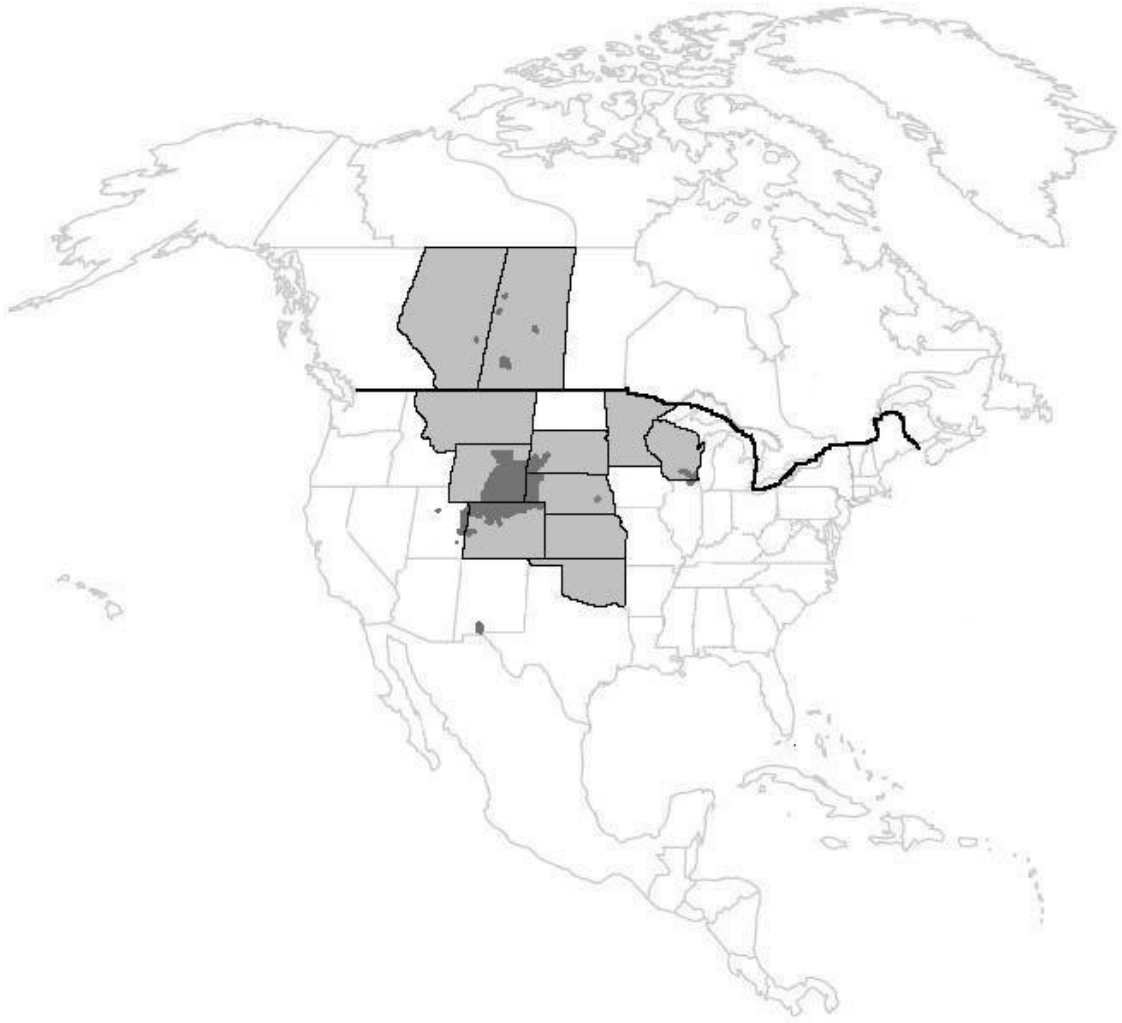

Fig. 2

Current distribution of CWD in farmed and free-ranging cervids in North America

Light gray fields indicate states or provinces with farmed CWD-affected cervids, dark gray fields indicate areas with free-ranging CWD-affected cervids.

2.1. Geographical distribution

To date, CWD has been diagnosed in farmed elk in USA, states Wisconsin, Minnesota, South Dakota, Nebraska, Oklahoma, Colorado, Montana, Kansas, and in Canada, provinces Saskatchewan and Alberta (Miller and Williams, 2004; Williams *et al*., 2002). The occurrence of CWD in free-ranging cervids is currently allocated into a few regions (Fig. 2). The greatest regions are the endemic areas in northeastern Colorado, southeastern Wyoming, and southwestern Nebraska. Since the year 2000, the disease in free-living cervids has been increasingly detected outside the original CWD-endemic areas in Colorado and Wyoming. Geographically isolated foci of CWD are reported from Nebraska, South Dakota, New Mexico, Wisconsin, and Saskatchewan (Williams *et al*., 2002).

One infected elk intended for farming was exported from Saskatchewan, Canada to South Korea. This was the first case of CWD occurrence outside the North America (Williams *et al*., 2002). Later, 9 out of 72 deer imported from Canada developed the disease during habitation in Korean farms, but cohabitating Korean deer were not infected (Kim *et al*., 2005).

Up to now any case has been reported from Europe, although some European countries have conducted active surveillance program. In Germany, 7300 captive and free-

ranging red deer, fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*) were tested during years 2002–2005 with no signs of infection (Schettler *et al*., 2006). In the UK, totally 304 cervids including roe deer, fallow deer, red deer, and muntjac (*Muntiacus muntjak*) were tested. The trial failed to detect any prion disease in tested animals. In the year 2003, 72 cervids in Switzerland and 196 reindeer in Norway were tested negative for CWD (Sieber *et al*., 2004; Sigurdarson, 2004). In Belgium, more than 200 brain and spleen samples from roe deer were tested negative using IDEXX HerdCheck test kit. These results were immunohistochemically confirmed (De Bosschere *et al*., 2006). Similarly, 290 roe deer were tested in Italy using the same test with no positive case (Meloni *et al*., 2007).

3. Origin of CWD

The exact origin of the disease remains obscure, but 3 major hypotheses regarding the possible origin of CWD have been suggested (Williams, 2005). The first hypothesis says that CWD arose from a spontaneous conformational alteration or somatic mutation of the cervid PrP followed by a transmission to other individuals. The other hypothesis supposes that CWD could originate from currently undefined prion strain of unknown source (Salman, 2003; Williams and Miller, 2002). However, the most plausible hypothesis says that CWD originated from scrapie (Williams, 2005; Williams and Miller, 2002). For many years the scrapie disease occurred among sheep in the USA. It is possible that due to some unknown factors the causative agent of scrapie became able to infect mule deer and later became adapted to the other cervids (Williams and Miller, 2002; Race *et al*., 2002). In support of this hypothesis, it should be noted that the scrapie prion protein (PrP^{Sc}) experimentally transmitted to the elk by intracerebral inoculation produced brain lesions that were undistinguishable from lesions produced by CWD detected by histopathology or immunohistochemistry (Williams, 2005; Hamir *et al*., 2004). Moreover, cross-species conversion experiments *in vitro* showed that ovine PrP^{Sc} induced the conversion of cervid PrP^{C} to PrP^{CWD} with high efficiency (Raymond *et al*., 2000).

4. Clinical signs of CWD

Clinical signs of CWD-affected cervids in terminal stage are similar to those in scrapie-affected sheep or cattle with bovine spongiform encephalopathy (BSE). However, alterations of locomotion activity can be unnoticeable in CWD-affected animals, in contrast to scrapie-affected sheep and BSE-affected cattle (Williams, 2005). The most prominent clinical features of CWD-affected animals are behavioral changes and progressive loss of body condition (Williams and Miller, 2002). The clinical course may last from a few weeks to a year and death typically occurs within four months (Williams and Miller, 2002). Behavioral changes are variable and may include personality alterations, behavior towards handlers, nervousness, and increased excitability. Repetitive move, lowered head, ear carriage, and periods of somnolence or depression may be noted. In most cases, a weight loss is an early clinical sign of CWD appearing shortly after the initial behavioral changes (Williams and Miller, 2002). In some cases polyuria, polydipsia, hypersalivation, esophageal dilatation with subsequent regurgitation, various nervous signs, such as lack of proper coordination, and posterior ataxia might be present during progression of the disease (Williams, 2005; Williams and Miller, 2002). Polydipsia and polyuria are probably associated with the damage of the supraoptic and paraventricular nuclei, where antidiuretic hormone is produced (Williams and Young, 1980).

Aspiration pneumonia occurring in the clinical course of CWD could be responsible for a rapid death and probably follows after a loss of the effective motor control over swallowing, what is associated with regurgitation (Williams, 2005; Williams and Young, 1992).

4.1. Histopathology

The particular histopathological lesions are observed in the central nervous system of CWD-affected animals. The presence of similar lesions was detected in every one of naturally susceptible host species (mule deer, white-tailed deer, and Rocky Mountain elk) (Williams and Miller, 2002). Histopathological lesions observed in the brains of CWD-affected animals are similar to those described in animals affected with scrapie or BSE (Williams, 2003; Williams and Young, 1993).

Histopathological lesions are characterized by spongiform transformation (microcavitation) of the grey matter, neuronal intracytoplasmic vacuoles, loss and degeneration of neurons, hypertrophy and hyperplasia of astrocytes with absence of inflammatory response. In all animals with clinical signs of CWD, there are histopathological lesions in the olfactory tubercle and cortex, hypothalamus, and parasympathetic vagal nucleus. The primary region in the brain, where spongiform changes could be detected by immunohistochemistry, is the parasympathetic vagal nucleus (Williams and Young, 1993). In addition, spongiform changes may be present in other parts of the brain, particularly in thalamus and cerebellum. The lesions in the cerebral cortex, hippocampus, and basal ganglia are generally mild (Williams and Miller, 2002; Williams and Young, 1993).

Ultrastructural observations show extensive membrane-bound vacuolation in neuronal processes, prominent astrocytic gliosis, dystrophic neuritis, and amyloid plaques (Guiroy *et al*., 1993). Amyloid plaques often surrounded by vacuoles containing CWD-associated prion protein (PrP^{CWD}) are common and can be detected on hematoxylin/eosin-stained brain sections of all natural hosts (Williams, 2005). Amyloid plaques are detected more easily with silver stains or Congo red staining followed by observation in polarized light (Williams and Young, 1993; Bahmanyar *et al*., 1985). Immunohistochemical detection of PrP^{CWD} using polyclonal or monoclonal antibodies can unambiguously reveal the presence of amyloid plaques.

In addition, PrP^{CWD} was detected in eyes, peripheral lymphoid nodes, gastrointestinal tract-associated lymphoid nodes, Peyer's patches, spleen, thymus, (Spraker *et al*., 2002) and skeletal muscles by the immunohistochemical analysis (Angers *et al*., 2006).

4.2. Pathogenesis

Oral transmission of CWD studied in mule deer showed that PrP^{CWD} appears primarily in lymph nodes, tonsils, and Peyer's patches (Sigurdson *et al*., 1999). From lymphoid tissues the PrP^{CWD} proceeds via sympathetic and parasympathetic nerves to the spinal cord and parasympathetic vagal nucleus in medulla oblongata, the initial sites of PrP^{CWD} accumulation in the brain (Williams and Miller, 2002). It was noted that pattern of PrP^{CWD} distribution in the brain is very similar to the distribution PrP^{Sc} in sheep brain (van Keulen *et al*., 2000).

It was suggested that PrP^{CWD} is spread by the follicular dendritic cells to lymphoid tissue and central nervous system (Sigurdson *et al.*, 2001; Sigurdson *et al*., 1999). The cells accumulating PrP^{CWD} are found within germinal centers of lymphoid follicles. Six weeks after oral inoculation of mule deer with CWD brain homogenate, PrP^{CWD} was mainly detected in extracellular association with follicular dendritic cell and B-cell membranes. It is assumed that the follicular dendritic cells can convert PrP^{C} into PrP^{CWD} at the cell membrane (Sigurdson *et al*., 2002).

4.3. CWD diagnosis

Clinical symptoms are commonly used for the detection of CWD-affected animals in captivity or in the wilds. However, the clinical signs are not strictly indicative of CWD and the recognition of CWD-affected animals may be rather uncertain, even experienced personnel may fail to detect individuals with clinical CWD.

Histopathology is one of the classic diagnostic techniques that are reliable. The samples collected from animals with CWD symptoms were examined for changes typical for CWD as microcavitation of grey matter, neuronal vacuolation, neuronal loss and degeneration, and astrocytosis. The most suitable tissue for histological examination is parasympathetic vagal nucleus from obex region in medulla oblongata, where histological changes occur commonly at the early stages of the disease (Williams and Young, 1993). Disadvantage of the histopathology for diagnosis include the requirements for very fresh tissues, which are not always available from field specimens.

Immunohistochemical examination is suitable for detection of PrP^{CWD} in the brain or lymphoid tissue before onset of the histological changes (Peters *et al*., 2000). Moreover, lightly autolysed samples can be also examined by this technique. Immunohistochemistry is very sensitive and specific method that can be used for testing of living animals, if tonsilar biopsy is performed (Wild *et al*., 2002). Main disadvantages of this method are time consumption, requirement of specialized skills, and possible subjectivity in the results interpretation.

Western blot analysis can be used for PrP^{CWD} detection in samples of the brain or lymphoid tissues collected from symptomatic or pre-symptomatic animals. (Spraker *et al*., 2004).

Commercially available CWD tests (Table 1) are designed at the same principles as BSE tests. ELISA based TeSeE® testing kit from Bio-Rad is the company's second-generation kit. It runs on an automated robotics platform that speeds up sample preparation enabling laboratories to provide results faster. Technicians using this testing platform can process up to 1000 samples per day with the same sensitivity as with immunohistochemistry. United States Department of Agriculture (USDA) approved the test for CWD testing in 1993.

Prionics®-Check PrioSTRIP test (Prionics) is used for routine CWD diagnostics in the USA and Canada during hunting season. After proteinase K digestion, the brain homogenate is incubated with color latex particles with bound monoclonal antibody. Detection is provided by strips that are submerged into the sample mixture. Antibody-prion protein complexes migrate up the strips and are bound at the test line. Free, unbound antibody binds at the control line. The result is interpreted immediately after the incubation, either visually or automatically.

Another strip-based test is PDL Rapid CWD Antigen Test (Prion Development Laboratories). The sensitivity of the test is about 99% and specificity 96%. Advantages of the test represent very fast results and no special accessories. The test was approved for white-tailed deer testing by USDA.

Idexx HerdCheck test (Idexx Laboratories) applies special polymer, Serpion ligand" that specifically binds to PrP^{CWD}. The bound PrP^{CWD} is detected with peroxidase-conjugated monoclonal antibody by ELISA. The test is fast and does not require proteinase K digestion. It was approved by USDA for white-tailed deer testing.

Table 1. Most frequently used tests for diagnosis of CWD

Test/manufacturer	Technique	Comments
TeSeE/Bio-Rad Laboratories	Sandwich ELISA	Full automation is possible, approved by USDA in 1993.
Prionics®-Check PrioSTRIP/Prionics	Lateral flow immunoassay with two MAbs	Used for routine CWD diagnostic during hunting season.
PDL Rapid CWD Antigen Test/Prion Development Laboratories	Lateral flow strip test	Simple test, approved for CWD testing in white-tailed deer.
IDEXX HerdCheck CWD Antigen EIA test/IDEXX Laboratories	Antigen capture ELISA	Utilizes special PrP^{CWD} binding polymer. Fast test without PK digestion. Approved for mule- and white-tailed deer.
CWD Dot Blot ELISA/VMRD Inc.	Dot blot ELISA	Used MAb, approved for mule- and white-tailed deer testing.
Check WESTERN test/Prionics	Western blot	Used as a confirmatory test.
Check LIA/Prionics	Luminescent sandwich ELISA	Compatible with Check Western test.
Prion Protein Detection Kit/Ventana IHC Systems and VMRD Inc.	Automated IHC staining of brain or lymphoid sections	Official APHIS test for CWD surveillance in captive cervids.
Enfer TSE test/Abbott Laboratories	Chemiluminiscent ELISA	Rapid screening test, fewer than 4 hrs to complete.

IHC = Immunohistochemistry; MAbs = monoclonal antibodies.

CWD Dot Blot ELISA runs in 96-well plate using one monoclonal antibody. The test was licenced by USDA for testing both mule deer and white-tailed deer.

Prionics-Check WESTERN Test detects PrP^{CWD} in brain stem homogenate using Westen blot analysis with monoclonal antibody. It is used for CWD diagnosis in mule deer and white-tailed deer. It is used frequently as a confirmatory test.

Prionics-Check LIA test is based on the chemiluminescent sandwich ELISA. The test is compatible with Prionics-Check WESTERN Test. Accordingly, the samples positive by the first test can be confirmed in the other one without the preparation of fresh samples.

Prion Protein Detection Kit (Ventana IHC Systems and VMRD) enables automated immunohistochemical staining of PrP^{CWD} in sections of the brain or lymphoid tissue with monoclonal antibody. It takes 3–5 days to complete the test depending on fixation time. It is the official APHIS (Animal and Plant Health Inspection Service) test for CWD surveillance in the captive cervids.

5. Transmission of CWD

It is broadly accepted that CWD is possibly the most efficiently transmitted disease of the mammalian prion diseases. In the free-ranging populations of cervids, prevalence of CWD varies between 1–30%, but in captive animals it can reach nearly 100% (Williams, 2005). Although CWD is an infectious disease, the understanding of the CWD transmission is still incomplete and probably occurs by both horizontal and vertical way. Horizontal transmission is the most prominent route of the CWD spread. Vertical (maternal) transfer cannot be excluded, but its role is not considered as important in the epidemiology of the disease (Miller and Williams, 2003; Miller *et al*., 2000). Since the PrPs of all TSEs are extremely resistant in the environment, the most likely route of CWD transmission is via prion-containing secretes and excretes (saliva, urine, and faeces) or decomposed carcasses (Williams *et al*., 2002). Environmental contamination appears to play a significant role in the spread of CWD mostly among captive animals. Contaminated pastures may serve as a source of CWD epidemics. The animals can be infected with PrP^{CWD} bound to soil particles, which are consumed by foraging (Miller *et al*., 2004). These observations are supported by the fact that prion particles strongly adhere to some soil compounds without the loss of infectivity and can persist there for 3 years (Johnson *et al*., 2006; Brown and Gajdusek, 1991).

Saliva is the potential source of infectivity, too. In hamsters and sheep intracerebrally infected with scrapie, the PrP^{Sc} are transported from brain to the tongue serving as a large potential reservoir for continual PrP^{Sc} shedding to saliva (DeJoia *et al*., 2006; Casalone *et al*., 2005). Likewise, the presence of PrP^{CWD} was confirmed directly in the deer saliva (Mathiason *et al*., 2006).

5.1. Experimental interspecies transmission of CWD

Mule deer, white-tailed deer, and Rocky Mountain elk are naturally susceptible to CWD. To date, no natural transmission of CWD to other species than the cervids was reported. However, many species are susceptible to CWD after experimental intracerebral inoculation. CWD infection of the sheep with VRQ genotype (most susceptible to scrapie) by the intracerebral inoculation resulted in the

development of the disease. Susceptibility of domestic sheep to CWD by the oral exposure has not been studied (Williams, 2005).

Intracerebrally inoculated fallow deer amplified PrP^{CWD} originating from white tailed-deer and elk. Histopathological lesions of spongiform encephalopathy were not observed, but PrP^{CWD} was detected in tissues of the central nervous system by immunohistochemistry, Western blot, and two commercially available rapid diagnostic tests. PrP^{CWD} amplification in fallow deer was minimal in comparison to CWD-affected cattle (Hamir *et al*., 2008).

Cattle were experimentally inoculated intracerebrally, orally, and via cohabitation with CWD-infected mule deer. Intracerebrally inoculated cattle developed the disease, but with relative low efficiency (5 of 13 animals) with an incubation period 2–5 years (Hamir *et al*., 2005). However, the secondary passage of the cattle PrP^{CWD} led to 100% efficiency of the transmission together with the decrease of incubation period to16 months (Hamir *et al*., 2006a). Potential CWD transmission to sheep and cattle was tested on mice expressing sheep or bovine PrP (Tamgüney *et al*., 2006). However, no signs of disease were detected 500 days after intracerebral infection of experimental animals with brain homogenate from CWD affected cervids, although cervid PrP was several folds over-expressed in experimental mice.

Experimentally, CWD has been successfully transmitted to domestic ferrets (*Mustela putorius fero*). The ferrets were inoculated intracerebrally with PrP^{CWD} and 75% of them developed the disease after incubation period of 17–21 months. The second passage of ferret PrP^{CWD} decreased the incubation period to 8–9 months and the third passage decreased it to 5 months (Bartz *et al*., 1998). In another study, ferrets were infected with PrP^{CWD} by the intracerebral and oral routes. Intracerebrally inoculated ferrets developed neurological signs consistent with CWD in 15–20 months after inoculation. Upon the first passage of ferret-adapted PrP^{CWD}, the incubation period decreased to 5 months. The ferrets inoculated orally did not develop any signs of the disease, even after 31 months of observation (Sigurdson *et al*., 2008).

Primary intracerebral challenge of CWD in mink (*Mustela vison*), a natural host of another prion disease, transmissible mink encephalopathy, resulted in the clinical disease in 25% of the animals after 31–33 months of incubation. The affected mink showed spongiform vacuolation, astrocytosis within the central nervous system, and demonstrated immunoreactivity to PrP^{CWD} in brain, retina, and lymph node. However, the oral challenge with CWD-positive elk brain did not result in the clinical or microscopic abnormalities (Harrington *et al*., 2008). Out of tested non-human primates, CWD was experimentally transmitted to squirrel monkeys (*Saimiri sciureus*) by the intracerebral inoculation with mule deer brain homogenate (Marsh *et al*., 2005). The PrP homology between the source and recipient species is a factor controlling interspecies TSE susceptibility (Raymond *et al*., 2000). Data obtained from *in vitro* experiments showed that a number of wild and domestic species appeared to be resistant or less susceptible to the PrP^{CWD} infection than the natural hosts. It suggested that the low homology of different PrPs acts as a molecular barrier that controls the transmission of CWD from cervids to the non-cervid species (Raymond *et al*., 2000; Williams *et al*., 2002).

5.2. Potential transmission to humans

Human susceptibility to the CWD is still unclear. Nevertheless, a few cases of Creutzfeldt-Jakob disease in people used to consume venison were reported in USA (Belay *et al*., 2004; Belay *et al*., 2001). However, no evident association between venison consumption and the development of the disease has been found. A possible transmission of CWD to humans was examined by the transgenic mice model. The mice expressing human PrP did not develop any symptoms of TSE after intracerebral inoculation of PrP^{CWD} during the lifespan (Zheng *et al*., 2007; Tamgüney *et al*., 2006; Kong *et al*., 2005). There is rather evidence of a substantial species barrier between cervids and humans that may strongly limit the transmissibility of PrP^{CWD} to the humans. The intracerebral route of TSE transmission is much more effective than the oral route (Prusiner *et al*., 1985). Since the most likely route of CWD transmission to the humans is through oral consumption of CWD-contaminated meat, the failure to detect transmission after intracerebral inoculation PrP^{CWD} in the transgenic human PrP-expressing mice suggests that the risk of CWD transmission to the humans is low (Kong *et al*., 2005).

In vitro experiments showed that PrP^{CWD} converts human PrP to pathological form very inefficiently. Bovine PrP was converted to its pathological form with low efficiency, but the ovine PrP was converted with moderate efficiency compared to the cervid PrP (Raymond *et al*., 2000). Probably the species barrier for cervid CWD transmission to humans is much more effective than that for the BSE transmission.

6. Cervid prion genetics

The deer and elk PrP primary structures are highly conserved similar to PrPs of other mammalian families. However, there are some polymorphic codons that may influence CWD susceptibility. The elk PrP polymorphic codon representing aa 132 (M/L) corresponds to the human PrP polymorphic codon 129 (M/V). The presence of elk 132MM and 132ML alleles were reported to be overrepresented among elk with CWD. Only one animal out of 226 CWD-affected elk was 132LL homozygote (Spraker *et al*., 2004).

Elk expressing PrP with 132LL allele experimentally inoculated with PrP^{CWD} did not develop the disease and seemed to be resistant to CWD (Hamir *et al*., 2006b). Similarly, the mice expressing elk PrP with aa 132L uniformly failed to develop the disease, while mice expressing elk PrP with aa 132M were consistently susceptible to CWD. In contrast, both strains of transgenic mice were susceptible to the scrapie. It was suggested that the elk aa 132 polymorphism controls prion susceptibility at the level of prion strain selection and that cervid PrP containing aa 132L severely restricts propagation of PrP^{CWD} (Green *et al*., 2008). In opposite to these findings, another experiment showed that all three genotypic variants of PrP with aa 132 (MM, M/L or LL) were equivalently susceptible to CWD. Among 47 CWD-affected free-ranging Colorado elk, the three genotypes of PrP were represented in the same proportion as in the group of healthy elk population (Perucchini *et al*., 2008).

Polymorphic codon corresponding to aa 225 (S/F) in deer PrP is believed to influence the susceptibility of mule deer to CWD. Among the CWD-affected mule deer the number of 225SS homozygotes is 30-fold higher than the number of 225SF heterozygotes (Jewell *et al*., 2005).

White-tailed deer PrP has polymorphic codon corresponding to aa 96 (G/S), which influence the susceptibility to CWD. It was found that the deer PrP 96S genotype significantly reduced susceptibility to CWD. PrP with 96S can affect the progress of the disease, as shown on transgenic mice expressing 96S PrP. These mice were completely resistant to the intracerebral CWD infection (Meade-White *et al*., 2007).

6.1. Elk PrP structure

The PrP structure of elk was examined using nuclear magnetic resonance analysis of recombinant elk PrP (Gossert *et al*., 2005). The global architecture of the various mammalian PrP structures is nearly identical. The full-length mature elk PrP (aa 23–231) is divided into two domains like PrPs of other mammals. N-terminal tail comprising aa 23–124 is flexibly disordered and the domain consisting of aa 125–226 has a globular structure. The C-terminal pentapeptide (aa 227–231) is flexibly disordered again. Elk PrP compared with other mammalian PrPs possesses well-defined loop of aa 166–175 connecting second α-helix and ß-sheet. Two aa exchanges in positions S170N and N174T were detected in the loop in comparison with bovine, human, and mouse PrP. It is supposed that this loop is a part of the disease-related epitope, which probably participates in the conversion of PrP^{C} to the pathologic form of PrP^{CWD} (Gossert *et al*., 2005). The inefficient transmission of CWD to other mammals could be explained by these significant aa mutations in elk PrP.

7. Conclusions

CWD is very effectively transmitted prion disease and is difficult to control the spread of disease in farmed cervids. However, the management of the disease in free-ranging animals is unfeasible. PrP^{CWD} persists in soil for several years and can be potentially infectious for domestic ruminants. Although no case of CWD in humans was reported and some indications for strong species barrier between cervids and humans exist, the casual transmission cannot be excluded. In spite of the fact that CWD was not reported from European countries, the systematic surveillance is needed to minimize the risk of uncontrolled spread of the disease.

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