CHAPTER TWELVE

Scrapie, CWD, and Transmissible Mink Encephalopathy

Candace K. Mathiason[1]
Colorado State University, Fort Collins, CO, United States
[1]Corresponding author: e-mail address: candace.mathiason@colostate.edu

Contents

Abstract

Transmissible spongiform encephalopathies (TSEs), or prions, are neurodegenerative diseases that affect a variety of animal species, including humans. Cruetzfeldt–Jakob disease (CJD) in humans, sheep and goat scrapie, chronic wasting disease (CWD) of cervids, and transmissible mink encephalopathy (TME) of mink are classified as TSEs. According to the "protein-only" hypothesis (Prusiner, 1982),[1] prions are devoid of nucleic acids and consist of assemblies of misfolded host-encoded normal protein, the prion protein (PrP^C). Prion propagation is thought to occur by a templating mechanism during which

Progress in Molecular Biology and Translational Science, Volume 150
ISSN 1877-1173
http://dx.doi.org/10.1016/bs.pmbts.2017.07.009

PrP^C is recruited, converted to a disease-associated isoform (PrP^D), and assembled onto the growing amyloid fibril. This fibular assembly is infectious, with ability to initiate disease processes similar to other pathogenic agents. Evidence indicates that scrapie, CWD, and TME disease processes follow this rule.

1. SCRAPIE

1.1 History and Epidemiology

Scrapie, the archetype transmissible spongiform encephalopathy (TSE), is a naturally occurring prion disease of sheep, goats, and mouflon (*Ovis musimon*).[2–4] Scrapie was first reported in Europe in the 16th century (England 1732 and Germany 1759),[5–7] with references to the disease in literature from the early Chinese and Roman epochs.[8] This is likely why the disease has been referred to by so many common names, including—shakings/shakers, cuddie trot, rida (ataxia or tremor), rickets, rubbers, goggles (to stare or squint), la tremblante (trembling), traberkrankheit (trotting disease), and prurigo lumbar (itchy back).[7–10] Scrapie has the distinction of being the first prion disease evidenced to be both infectious and transmissible in natural settings.[11,12]

In the early 20th century (between 1920 and 1958), scrapie became an economical concern for sheep breeders. Most sheep breeds have been affected by scrapie, although it is more common in some breeds than others. International trade of Suffolk sheep incubating the disease lead to the introduction of scrapie in numerous countries, resulting in endemic infections in flocks around the world.[9,13–15] Progress has been achieved in the prevention of scrapie in sheep due to efficient genetic breeding programs based on eradication and control of the disease. In general, the selection plans for sheep aim to eliminate and reduce the susceptible alleles (VRQ) and to enrich the resistant allele (ARR).[16]

The codons of the prion protein (*Prnp*) that are especially significant in determining susceptibility or resistance to classical scrapie in sheep include 136, 154, and 171[17] and for atypical sheep scrapie include 141, 154, and 171.[18] *Prnp* haplotypes correlated with increased susceptibility are 136 valine (V), 154 arginine (R), and 171 glutamine (Q) (VRQ), whereas 136 alanine (A),[17,19,20] 154 histidine (H),[21] and 171 arginine (R)[22,23] are associated with resistance to natural scrapie. Codon 171 appears to have the most discernible influence, where sheep with 171 QQ are susceptible and 171RR are resistant. Goat scrapie has been less well studied. A number of polymorphisms

appear to be associated with goat scrapie susceptibility and resistance.[24] While none confer complete susceptibility or resistance, K222 has been suggested as a good candidate for selective breeding programs to enhance scrapie resistance in goats.[25,26]

Natural scrapie in sheep and goats occurs in classical and atypical forms, which are distinguished on the basis of neuropathology and PrP^{Sc} glycosylation patterns on western blot.[27,28] Atypical scrapie was first reported in 1998, and has now been identified throughout Europe, mainly through active surveillance of asymptomatic sheep.[29–31] The earliest evidence for atypical scrapie in sheep dates back to 1972.[32] Atypical scrapie tends to occur in older sheep with *Prnp* genotypes considered resistant to classical scrapie (ARR/ARR).[33–35] Both forms (classical and atypical scrapie) have been demonstrated in the same naturally infected animal,[32,36,37] giving rise to questions regarding the origin of atypical scrapie. Experimental inoculation studies have suggested that the phenotype of atypical scrapie can in some instances change into that of a second strain during passage in sheep.[38] It is therefore more likely that natural TSE infections of ruminants involve mixtures of strains rather than a single strain.[32]

1.2 Clinical Signs

Classical and atypical scrapie can be distinguished by careful observation of clinical signs.[39,40] Clinical signs of classical scrapie include hunched posture, hind limb ataxia with fore limb hypermetria, scratching, and wool loss.[41,42] Whereas sheep with atypical scrapie exhibit normal posture, circling, no scratching, ataxia, visible head tremor, and no wool loss.[40] Classical scrapie is typically found among sheep between 2 and 5 years of age[42–45] and in goats >6 years of age[46] with no gender bias.

1.3 Distribution of Scrapie in Sheep Tissues

In naturally acquired scrapie, evidence suggests that the main route of entry of the infectious agent is through the gut-associated lymphoid tissues (GALTs) with subsequent spread to the central nervous system (CNS) by centripetal transport along the peripheral nervous system (PNS).[47,48] A large body of evidence indicates that scrapie infection ascends from the gut into the thoracic spinal cord and to the medulla oblongata along the splanchnic and vagus nerves. From these initial sites, infection propagates cranially and caudally within the CNS, with subsequent centrifugal spread to the sensory ganglia of the vagus and splanchnic circuits.[49] Further

centrifugal transport from the brain to peripheral tissues appears to be along the PNS.[50–53] It is widely recognized that the lymphoreticular system plays an important role in the dissemination of the prion agent.[54] Infectivity and/or PrP^{Sc} have been demonstrated in peripheral tissues of natural scrapie infections, including placenta,[55,56] skeletal muscle,[52,57] pancreas,[52,58] kidney,[52,59,60] liver,[52,61] adrenal glands,[52,62,63] heart,[52] urinary bladder,[52] lung,[52] mammary gland,[52,64] and skin,[52,65] suggesting that the agent may propagate in peripheral organs.[52] The detection of PrP^{Sc} deposition in sheep has, in some cases (mammary gland and liver), been found in combination with the presence of an inflammatory response to another pathogen.[52,66]

Tissue distribution of the scrapie agent may be influenced by several factors including host genetics, dose, and strain.[47,52] For example, sheep with resistant vs susceptible *Prnp* genotypes develop natural scrapie with longer incubation periods and less or varied dissemination of PrP^{Sc} in CNS and PNS tissues.[52,67–69]

1.4 Diagnosis

Diagnosis of sheep scrapie can be achieved via a combination of clinical observation and detection of the biomarker associated with the disease, PrP^{Sc}, in lymphoid biopsy or brainstem at the level of the obex.[70] Biochemical methods for differentiation of classical and atypical scrapie are available. Atypical scrapie PrP^{Sc} is more sensitive to proteinase K (PK) digestion than classical scrapie PrP^{Sc} and has a more variable pattern on western blots, including a characteristic low molecular mass band (estimated at 7–12 kDa) not present in classical scrapie.[71] To complicate detection further, infectivity has been demonstrated in peripheral tissues of sheep with atypical scrapie devoid of PrP^{Sc},[72] suggesting that atypical scrapie may at times be difficult to detect by conventional methodologies used to surveille for scrapie.

1.5 Transmission and Pathogenesis

An increase in scrapie incidence has long been associated with the lambing season,[42] so it is thought that most animals are infected at birth or shortly thereafter[42] via contact (presumed oral consumption)[73–76] with prions in placental tissues known to contain prion infectivity[76–79] or infectious prions shed into the environment.[80] Prion infectivity has also been demonstrated in the blood,[58,81–83] milk, colostrum,[84–86] saliva,[87] and environments[45,88,89] of scrapie-infected sheep and goats. The demonstration of prion infectivity in

pregnancy-related milieu (milk, colostrum, and placenta) and maternal blood suggests that maternal/vertical transmission plays an important role in early scrapie exposure. The recent demonstration of in utero scrapie transmission in sheep during clinical and preclinical stages of disease provides additional evidence to this point.[90] Scrapie transmission dynamics and efficiency would likely rival that of chronic wasting disease (CWD) in cervid species without the advent of breeding programs to select for genotypes resistant to scrapie infection.

1.6 Zoonotic Potential

Early epidemiological studies lacked support for a causal relationship between sheep scrapie and human infections,[91] and to date there is no evidence for an increased zoonotic risk in the human population. Experimental studies provide conflicting results as to the zoonotic potential of scrapie. Some studies have failed to demonstrate transmission of scrapie to nonhuman primates[92] and transgenic mice overexpressing the human prion protein.[93] Other studies, using natural classical and atypical ovine scrapie strains and marmoset,[94] transgenic mice,[95] or cynomolgus macaques[96] have provided reason to revisit this premise. Cynomolgus macaques, a highly relevant model for human prion disease, demonstrated susceptibility to infection by a natural classical scrapie isolate after a 10-year silent phase of disease.[96] These findings require additional studies to assess the risk natural scrapie strains pose to human public health.[97]

2. CHRONIC WASTING DISEASE

2.1 History and Epidemiology

CWD is unique among prion diseases, being the only TSE to infect free-range and captive wildlife populations. CWD has to date been identified in mule deer (*Odocoileus hemionus*), black-tailed deer (*O. hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), moose (*Alces alces*), and reindeer (*Rangifer tarandus*). The geographical distribution of CWD (Fig. 1) continues to expand, now including 24 states and 2 Canadian provinces of North America,[98] transport from North America to South Korea,[99,100] and most recently the first cases have been identified in Europe (Norway 2016).[101] The disease syndrome of wasting and eventual death was first observed in cervids held in captivity in Colorado and Wyoming in the late 1960s. It was initially thought to be the result of nutritional

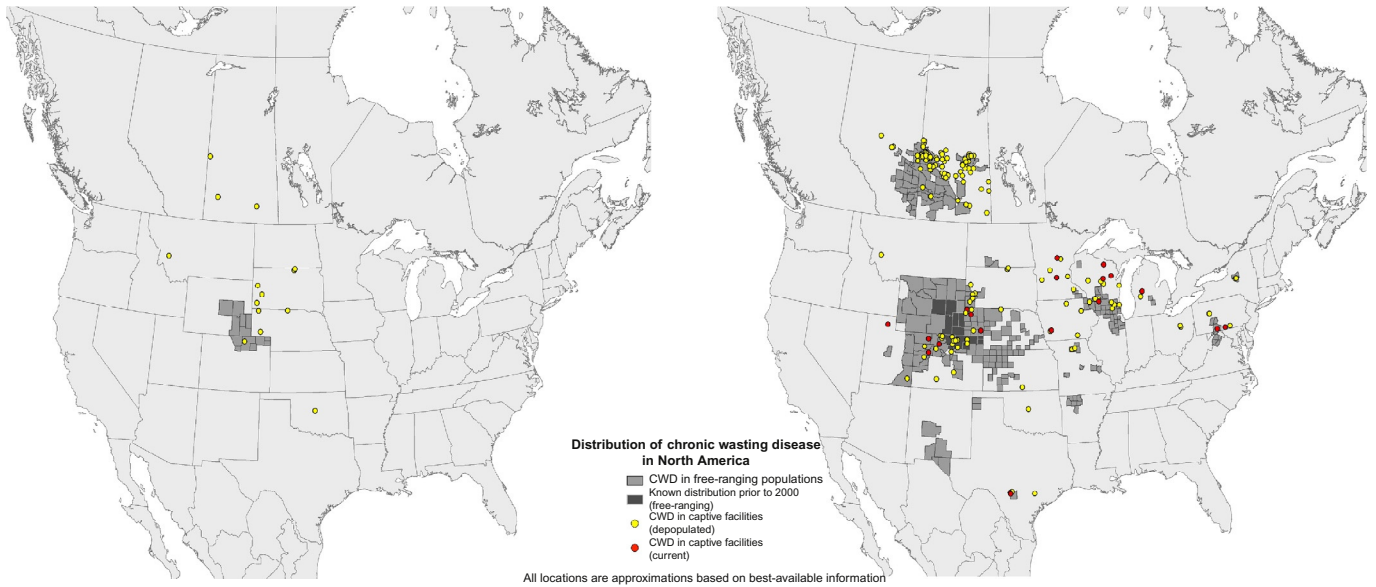

Fig. 1 Distribution of CWD in North America. *Maps provided by Bryan Richards, USGS.*

deficiency/or stress associated with their confinement. The disease was described as a spongiform encephalopathy in 1980[102] and was soon after identified in free-range elk and mule deer.[103,104] CWD is the most efficiently transmitted of the prion diseases. Free-range prevalence rates are reported to be <5% in deer and <2.5% in elk, with hot spots as high as 50% in free-range populations and >90% in captive facilities.[105] To date there is no clear evidence to explain the origin of CWD. Suggested hypotheses include the transmission of sheep scrapie to cervids, or a spontaneous conversion of the normal cervid prion protein to a misfolded confirmer that was transmissible between cervid species. Prevalence rates between males and females in free-range populations are variable dependent upon location; a slight increase in males,[106] twofold higher rates in males to females,[107] or nearly twice the infection rate in females as males.[108]

Deciphering the genetic susceptibility of cervids to CWD has been pursued. The structure of the mature prion protein is consistent across taxa, with a long, flexible N-terminal tail, three alpha-helices and a two-stranded antiparallel beta-sheet.[109] An amino acid loop connecting the beta-sheet strand to the alpha-helices is particularly rigid in cervids.[110] This rigid loop structure appears to be influential in the efficiency of PrP^{C} to PrP^{CWD} conversion[111] and may have bearing on the highly infectious nature of CWD in cervids.[110–112] Genetic analysis of CWD-affected cervid populations suggests that *Prnp* variation affects susceptibility and/or disease progression. Many of these conclusions have been based upon the proportion of affected and unaffected cervids in a population with respect to their *Prnp* genotype. Studies have evaluated disease susceptibility and progression of *Prnp* genotypes based on tissue pathogenesis, infection rates in captive animals, disease incubation period in experimentally challenged animals, and determination of genotype-specific infection and survival rates in native free-range populations. These studies in elk, mule deer, white-tailed deer, moose, and reindeer have revealed some correlations between *Prnp* sequence and CWD infection.

2.1.1 Elk

Studies in Rocky Mountain elk have found that polymorphisms at *Prnp* codon 132, encoding either a methionine or leucine may influence the susceptibility to CWD infection.[113,114] An initial report found that there was an overrepresentation of 132MM *Prnp* genotype in CWD-infected elk.[115,116] Further survey of free-ranging elk in Colorado found evidence for CWD infection in 132MM, 132ML, or 132LL in proportion to their frequency

in the population.[117] Experimental oral inoculation studies in elk demonstrated modulation of incubation time dependent upon 132 expression, i.e., 132MM expressing elk exhibit the shortest time to clinical disease (23 mpi), followed by 132 ML at 40 mpi, with the longest incubation periods being associated with 132LL (60 mpi).[118,119]

2.1.2 Mule Deer and White-Tailed Deer

Mule deer carry a unique polymorphism at codon 225, serine (S), or phenylalanine (F).[120,121] While lower prevalence rates, lengthened incubation time,[122] and delayed PrP^{CWD} tissue accumulation[123] have been shown in 225SF genotype mule deer, complete resistance is not conferred. Studies of the white-tailed deer *Prnp* genotype have also been conducted. These studies have found two coding polymorphisms, Q95H and G96S, which occur at lower than expected frequencies in CWD-infected animals.[124–128] The serine substitution for glycine at residue 96 (S96G) may be associated with slower disease progression, but neither S96G nor G96G confer resistance to CWD infection.[125,129] Further investigation of the affects *Prnp* gene haplotypes/diplotypes may have on CWD resistance has been applied to a population of white-tailed deer in Wisconsin/Illinois. Results from this survey suggest that deer populations with higher frequencies of haplotype C or diplotypes AC and BC might have a reduced risk for CWD infection.[130]

2.1.3 Moose and Reindeer

Few free-range CWD-infected moose has been identified,[131] and the first natural infection of reindeer has been reported.[101] The homozygous 209MM amino acid sequence was identified in the index case of wild moose CWD, but subsequent experimental studies designed to further define CWD resistance in moose have demonstrated susceptibility in both 209MM and 209IM moose.[132] The *Prnp* genetic sequence in the first reindeer naturally infected with CWD[101] was found to be identical to that found in two experimentally infected reindeer (homozygous V (valine), G (glycine), S (serine), and V (valine) at codons 2, 129, 138, and 169, respectively).[133]

In summary, none of the cervid *Prnp* genotypes described are highly diverged from the known susceptible genotypes, suggesting that *Prnp* divergence alone is unlikely to provide complete resistance to CWD infection. Thus, a population of cervid with prolonged infectious states could contribute to environmental contamination and subsequent transmission to

susceptible animals in captive and native free-range cervid populations[134,135] and may be driving the decline of some cervid species.[108]

2.2 Clinical Signs

Disease presentation in wild and captive populations is initially recognized as progressive weight loss and isolation from the herd population. As disease progresses infected cervids exhibit excessive salivation, polydipsia, polyuria, bruxism, regurgitation, ataxia, and tremors, with terminal disease presentation within 18–24 months.[136] Experimental inoculation studies have provided further observation of clinical disease progression, with similar time to terminal disease (~24 months postexposure).[137,138] The earliest signs of clinical disease in white-tailed deer inoculated with brain (oral), blood (intravenous or intraperitoneal), or saliva (oral) from CWD-infected cervids included behavioral changes (isolation, increase/decrease response to human interaction) and were seen between 12 and 20 months postinoculation. Clinical disease onset manifested primarily as perceived body muscle-mass reduction and gradual weight loss, which reached $\geq$20% of maximum body weight over 2–8 months. Other late-stage clinical signs included: rough hair coat due to piloerection and a body stance characterized by a low head position and wide leg stance. Changes in behavior included hyperphagia and polydipsia despite weight loss, and stereotypic movements including head tossing, repetitive and exaggerated lifting of the legs, diminished alertness, and occasional aggressive behavior in the advanced stage of disease.[137,139]

2.3 Distribution of CWD in Cervid Tissues

The distribution of CWD in cervid tissues has been investigated via microscopy (immunohistochemistry—IHC) and in vitro amplification methodology (real-time quaking-induced conversion—RT-QuIC).

2.3.1 Histopathology

The terminal brain histopathology of CWD-affected animals is largely congruent across cervid species.[101,103,140–142] Lesions within the central nervous tissue are associated with gray matter with similar severity in natural and experimental infections. Bilateral lesions including spongiosis in neuronal perikarya and neuronal processes, astrocytic hyperplasia, and hypertrophy are apparent in brain tissue of infected cervids. Florid amyloid plaques, pale fibrillar esoinophic areas of neuropil surrounded by vacuoles, are common in brain tissues.[103,142,143] Histopathology has limited practical use in assessing

preclinical status of disease progression in free-range populations due to obvious inaccessibility to brain tissue biopsies or serial access to lymphoid biopsies to monitor CWD status. However, it has been extensively used to assess PrP^{CWD} accumulation and disease dynamics in experimental studies.

2.3.2 Pattern of PrP^{CWD} Tissue Accumulation

Oral exposure is the most plausible pathway by which CWD prions are introduced to deer in nature. Thus, longitudinal experimental studies conducted to emulate this presumed mode of transmission provide insight into the patterns of PrP^{CWD} tissue deposition.[123,144] Accumulation of PrP^{CWD} was detected as early as 42 dpi in follicular germinal centers of the retropharyngeal lymph nodes, Peyer's patches and ileocaecal nodes, and at 78 dpi in tonsilar tissue of orally exposed fawns.[144] A subsequent experimental study incorporating the same CWD inoculum, dose, and oral exposure in mule deer fawns provided extended analysis of PrP^{CWD} tissue deposition from 90 dpi to terminal clinical CWD disease (630–785 dpi).[123] PrP^{CWD} accumulation was observed in the GALT and ganglia of the enteric nervous system, as well as the dorsal motor nucleus of the vagus nerve (DMNV), the intermediolateral column of the spinal cord, and the vagus nerve. From there, PrP^{CWD} accumulation was detected in the rest of the brain initially affecting nuclei in the medulla, thalamus, hypothalamus, midbrain, and olfactory cortex.[123] The general progression of PrP^{CWD} accumulation was characterized by rapid and widespread involvement of lymphatic tissues followed by the tissues of the CNS and PNS, and the endocrine system of animals exhibiting terminal clinical CWD.[123] Assessment of white-tailed deer tissues harvested prior to 4 months postinfection by the RT-QuIC methodology corroborates initial prion detection in pharyngeal lymphoid tissue between 30 and 60 dpi and supports systemic dissemination prior to neuroinvasion.[145]

2.4 Diagnosis

The current United States Department of Agriculture Animal and Plant Inspection Service (USDA-APHIS) approved test for the CWD herd certification program for farmed or captive cervids is immunohistochemistry and Bio-Rad ELISA.[146] The western blot is also an official test when performed at certified laboratories. All suspect positive Bio-Rad ELISA test and suspect positive IHC test results are confirmed by the National Veterinary Services Laboratory.

Several peripheral lymphoid tissues have been evaluated for their ability to permit antemortem diagnosis of CWD. The interpretation of tests for CWD is complicated by a long incubation period, the pattern of distribution of prions throughout the body, and the influence of genetics on the progression of disease in infected animals. As a result, a truly infected animal may not have detectable prions in sampled tissue at the time of testing. In addition, the antemortem biopsy collection provides a smaller sampling of the target tissue than is evaluated postmortem, enhancing the feasibility that prion deposition may be missed. Together, these factors have implications for CWD live-animal testing that are very different than when assessing CWD status at the time of necropsy. Thus, CWD antemortem testing has important regulatory implications if used to permit the interstate movement of animals and/or release quarantines in CWD-infected or exposed herds, i.e., testing may fail to detect infected animals in the preclinical phase of disease. Additional diagnostic tools are critically needed to manage CWD.

The development of assays with sensitivity and specificity to detect prions during the protracted asymptomatic phase of disease is ongoing. Two such assays, protein misfolding cyclic amplification (PMCA)[147] and RT-QuIC,[148,149] have made considerable contribution to the understanding of CWD distribution, pathogenesis, and transmission dynamics.[134,145,150–161]

2.5 Transmission and Pathogenesis

How CWD is transmitted from one susceptible cervid to the next has been an ongoing mystery. Direct animal-to-animal contact and indirect exposure to CWD prions in the environment are thought to be the most probable modes of CWD transmission within free-range cervid populations.[162] Experimental studies have helped to define the point source of CWD infectivity that is presumed to account for horizontal transmission. Secretions and excreta (saliva, urine, and feces) from cervids infected with CWD have been shown to harbor infectivity.[139,153,163,164] Of particular concern is the finding that infectivity may be shed by infected animals throughout the protracted preclinical phase of disease that can last months to years.[164] The shedding of infectivity throughout the full disease course likely enhances exposure risk to CWD—especially as herd density and environmental prion load increase during winter pasturing. Further contribution to environmental contamination includes carcasses of infected animals, as tissues[165] including skeletal muscle,[166] antler velvet,[167] and blood[139,168] contain infectivity. CWD prions are known to retain infectivity in the environment for at least

2 years.[162] In addition, CWD infectivity can be transferred with fomites (buckets, hay, and water) establishing infection within 15–19 months post-exposure.[137] Maternal transmission may also play a role in the spread of CWD. Prions have been detected in fetal and reproductive tissues harvested from experimental[160] and naturally infected[169] cervids, and infectivity has been demonstrated within the pregnancy microenvironment of muntjac deer.[170]

2.6 Zoonotic Potential

Strong evidence of zoonotic transmission of bovine spongiform encephalopathy (BSE) to humans has led to concerns about zoonotic transmission of CWD. As noted above, CWD prions are present throughout the diseased host, including muscle, organs, blood, antler velvet, and tissues of the CNSs and PNSs. Exposure to—and consumption of—materials from CWD-infected cervids places humans at increased risk. To date there has been no known transmission of CWD to humans and the zoonotic potential is thought to be low based on experimental studies using Cynomologus macaques,[171] transgenic mice overexpressing human prion protein,[172] and in vitro assays showing low efficiency of PrP^{CWD}-directed conversion of the normal human prion protein to the aberrant disease-associated form.[173] However, squirrel monkeys are susceptible to CWD by oral inoculation[171] and recent in vitro assays showing efficiency of PrP^{CWD}-directed conversion of the normal human prion protein at the level of the protein–protein interaction suggest the barrier preventing the transmission of CWD to humans may be less robust than previously estimated.[150] Clearly, additional research is needed to further investigate the zoonotic potential of CWD.

The role reservoir species may play in CWD transmission, including enhanced zoonotic potential, has also been under investigation. Interspecies transmission of CWD to noncervid species sympatric with CWD-infected cervids, including cattle,[174,175] raccoons, coyotes, and opposums[176] has not been observed under natural conditions. Experimental studies have provided evidence that several species, including voles, mink, ferrets, cats, goats, sheep, cattle, and mice are susceptible to CWD infection when exposed via intracranial inoculation.[138,175,177–180]

There is convincing evidence that CWD strains exist.[181] Disease course, including clinical presentation, incubation period, and prion deposition vary dependent upon prion strain.[171,182] As CWD continues to emerge, infecting additional susceptible cervids as well as new cervid species,[101] the potential

for strain divergence,[182,183] and interspecies events increases.[184] The distribution and prevalence of CWD strains in native cervid populations is unknown. As are factors associated with increased risk of interspecies transmission including the cervid and human genotypes that define interspecies transmission potential of CWD. The zoonotic potential of CWD, therefore, remains unknown.

3. TRANSMISSIBLE MINK ENCEPHALOPATHY

3.1 History and Epidemiology

Transmissible mink encephalopathy (TME) is an invariably fatal disease of ranched mink with similar clinical presentation and pathology as sheep scrapie.[185] It was initially identified in mink in 1947 in Wisconsin, United States, with subsequent outbreaks on five additional farms in Wisconsin in 1961.[186] TME has further been recognized on a farm in Ontario, Canada and two farms in Wisconsin that used feed from the same supplier. The most recent outbreak in North America was again in Wisconsin in 1985.[187] The disease has also been reported in Idaho, United States (1963),[185] Finland (1966),[185] East Germany (1967),[188] and the former Soviet Union (1979–early 1980s).[189]

The origin of TME is hypothesized to be the ingestion of prion-contaminated feed of ovine or bovine origin.[186,187] This hypothesis is supported by the occurrence of outbreaks on several farms using the same feed supply.[186] In the United States, tissues of cattle, mainly "downer" dairy cows, was commonly used in preparing mink feed and is now considered the likely source of the TME agent.[187] In the Soviet Union the origin of TME has been traced to feeding carcasses of scrapie-infected sheep to mink.[190] Further support for bovine or ovine-sourced encephalopathies as the origin of TME has been provided by experimental intracranial (IC) inoculation of TME into cattle[191] or sheep[192] that resulted in neurologic disease similar to BSE and scrapie, respectively. As well, the reverse, i.e., experimental IC or oral inoculation of BSE[193] or scrapie[194,195] into mink resulted in disease presentation similar to TME. Incubation times associated with clinical disease progression in these studies[196] and other cross-species transmission studies[197] have led to the suggestion that the etiology of TME is L-type BSE vs C or H-type BSE or sheep scrapie.

Male and female adult mink are affected equally with mortality rates ranging from 10% to 100%.[186] A study of the mink prion protein did not

identify *Prnp* polymorphisms that influence susceptibility, incubation period, clinical course, or neuropathologic pattern.[198]

3.2 Clinical Signs

Clinical disease in affected mink manifest in 7–12 months postexposure, with either a slow progressive disease process over a period of weeks (2–7 weeks) or a rapid progressive disease course lasting ~1 week. Changes in behavior include hyperexcitability, hyeresthesia, ataxia, whole body tremors, bouts of drowsiness, and increased aggressiveness.[199]

3.3 Distribution of TME in Mink Tissues

3.3.1 Histopathology

TME histopathological changes are characterized by spongiform alterations in the neuropil of the gray matter, neuronal degeneration, and astrycytosis.[185,189,200] The most obvious changes are spongiform alterations that vary from localized to diffuse neuropil vacuolation. Neuronal degeneration is marked by dark, shrunken angular neuronal perikarya. Astrocytosis in the gray matter is prominent, with reactive astrocytes appearing as enlarged, pale naked nuclei. Amyloid plaques are not found in TME.[199,200] Severe degenerative changes are present in the thalamus, amygdala, and caudal colliculi, with more moderate lesions in the hippocampus and hypothalamus. Lesions in the brain stem are generally less intense and more variable, with limited changes in the pons, medulla oblongata, and no affect on the cerebellum.[199]

3.4 Diagnosis

Behavioral changes in mink affected with TME may be mistaken for the effects of a neurotoxin or acute infection of the CNS.[201] Upon further progression of the disease (~1 week) it is easily distinguished from other naturally occurring neurodegenerative diseases of ranched mink. Microscopic examination of brain tissue from affected mink confirms a TME diagnosis.[192]

3.5 Transmission and Pathogenesis

TME is not a naturally transmitted disease, and therefore does not sustain itself in the mink population.[192,194] Transmission is associated with foodborne contamination events.[186,187] Further study suggests the origin of TME to be L-type BSE.[197]

Experimental TME transmission can be achieved by oral, intradermal, subcutaneous, intramuscular, intraperitonal, and intracerebral inoculation.[187–189,199,202,203] Incubation times vary, dependent upon route of inoculation and titer of inoculum, ranging from 4 to 12 months—IC, 4 months; oral 7–8 months. The experimental host range includes the domestic ferret, striped skunk, pine and beech martins, raccoons, Syrian and Chinese hamsters, squirrel, rhesus and stumptail macaque monkeys, sheep, goat, and cattle, with variable incubation times post-IC inoculation between 5 (skunk) and 65 months (sheep) pi.[191,203–210]

Isolation of hamster-adapted TME strains and development of their use to investigate TSE stain adaptability have provided considerable insight into the TSE strain selection process. Two TME strains producing distinctly different clinical syndromes and brain titers in Syrian hamsters, "drowsy" (DY) and "hyper" (HY),[183,211] were isolated, enriched, and demonstrate strain-specific conversion of PrP^{C} to PrP^{RES} in vitro.[212] Further use of these distinct TSE strains has resulted in demonstration that coinfection with the two strains could result in the DY TME phenotype and PrP^{RES} conformation on first passage, but upon subsequent passages, the disease pattern converted to HY TME.[213] These findings, and subsequent investigations,[214–222] indicate that during TSE strain adaptation, there is selection of a strain-specific PrP^{RES} conformation that can determine the TSE strain phenotype.

3.6 Zoonotic Potential

As TME disease is extremely rare the zoonotic potential to humans is negligible. Yet, TME has provided insight to cross-species transmission dynamics and their potential implications to future zoonotic events of prion diseases, including the possible presence of L-BSE for many decades prior to its identification in the United States and Europe.[197]

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