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Animal Diseases

REVIEW

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Classical bovine spongiform encephalopathy and chronic wasting disease: two sides of the prion coin

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Abstract

Transmissible spongiform encephalopathies (TSEs) are a group of progressive and ultimately fatal neurologic diseases of man and animals, all resulting from the propagated misfolding of the host's normal cellular prion protein. These diseases can be spontaneous, heritable, anthropogenic/iatrogenic, or in some cases horizontally transmissible, and include such notable TSEs as bovine spongiform encephalopathy (BSE) of cattle and chronic wasting disease (CWD) of cervids. Although they are both unequivocally protein misfolding disorders, they differ markedly in their pathogenesis, transmissibility, and zoonotic potential. While the BSE epidemic has largely abated over the past three decades following global feed bans on ruminant meat and bone meal, CWD, which is readily transmitted through various forms of excreta, has rapidly expanded from its original endemic zone to encompass much of North America, along with recently identified foci in Scandinavia. Most importantly, although the classical form of BSE has proven transmissible to humans consuming contaminated beef or beef products, so far there have been no conclusive reports on the zoonotic transmission of CWD to humans. The underlying basis for these differences – whether host or agent directed – are not well understood, though may be due to inherent differences in the three-dimensional structure of the misfolded BSE or CWD prion proteins or the expression levels and tissue distribution of respective cellular prion proteins. With the uncontrolled geographic spread of CWD, it is imperative that we improve our understanding of the factors governing prion disease pathogenesis, transmission, and zoonotic potential.

Keywords Prion, Bovine Spongiform Encephalopathy, Chronic Wasting Disease, Transmission, Pathogenesis, Zoonosis

Introduction

The transmissible spongiform encephalopathies, or TSEs, are a group of infectious agents that are responsible for progressive, degenerative, and uniformly fatal central nervous system (CNS) disorders in their respective animal hosts, including humans (Collins et al. 2004). These diseases, including bovine spongiform encephalopathy (BSE) of cattle, chronic wasting disease (CWD) of deer and other cervids, and human Creutzfeldt-Jakob disease (CJD), tend to be highly species-specific and share similar patterns of clinical disease, neuropathology, and etiology. The agent at the heart of all TSEs is a misfolded isoform of the normal cellular prion protein, generally referred to as PrP^{Sc} for the various forms of disease-causing prions, is capable of forming a highly stable and neurotoxic amyloid that is devoid of nucleic acids and other familiar genetic building blocks found in bacteria, viruses, and other conventional pathogens (Prusiner 1982).

Transmissible spongiform encephalopathies may arise and spread through one of several different mechanisms. Some prion diseases, including CWD, are highly

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infectious between susceptible species, with horizontal (Williams 2005), vertical (Selariu et al. 2015; Nalls et al. 2021), and environmental transmission mechanisms reported (Mathiason et al. 2009; Miller et al. 2004). Its highly infectious nature has led to an incredible expansion of CWD across North America over the past several decades (Fig. 1). In contrast, others the classical form of BSE (c-BSE) are, in most cases reported so far, anthropogenic, or in some cases iatrogenic, with little evidence of animal-to-animal, person-to-person, or environmental transmission (Smith and Bradley 2003). Once the basis of c-BSE transmission was identified – the supplementation of cattle feed with rendered cattle meat and bone meal contaminated with BSE prions – the epidemic of the late 1980s and early 1990s was brought to a grinding halt (Narang 1996). Over the past two decades, most of the newly arising BSE cases identified worldwide have been atypical forms, spontaneous in nature, with case numbers significantly lower than those reported in the c-BSE epidemic of the late twentieth century (European Food Safety Authority 2021). Aside from infectious, anthropogenic/iatrogenic, and spontaneous forms of disease, a fourth mechanism of prion etiology, namely heritable forms, seems to be of relatively minor importance for either CWD or BSE, though is an important form of human TSEs (Appleby et al. 2022). It is not well understood why prion diseases like CWD are horizontally transmissible, while others like BSE are not. Some

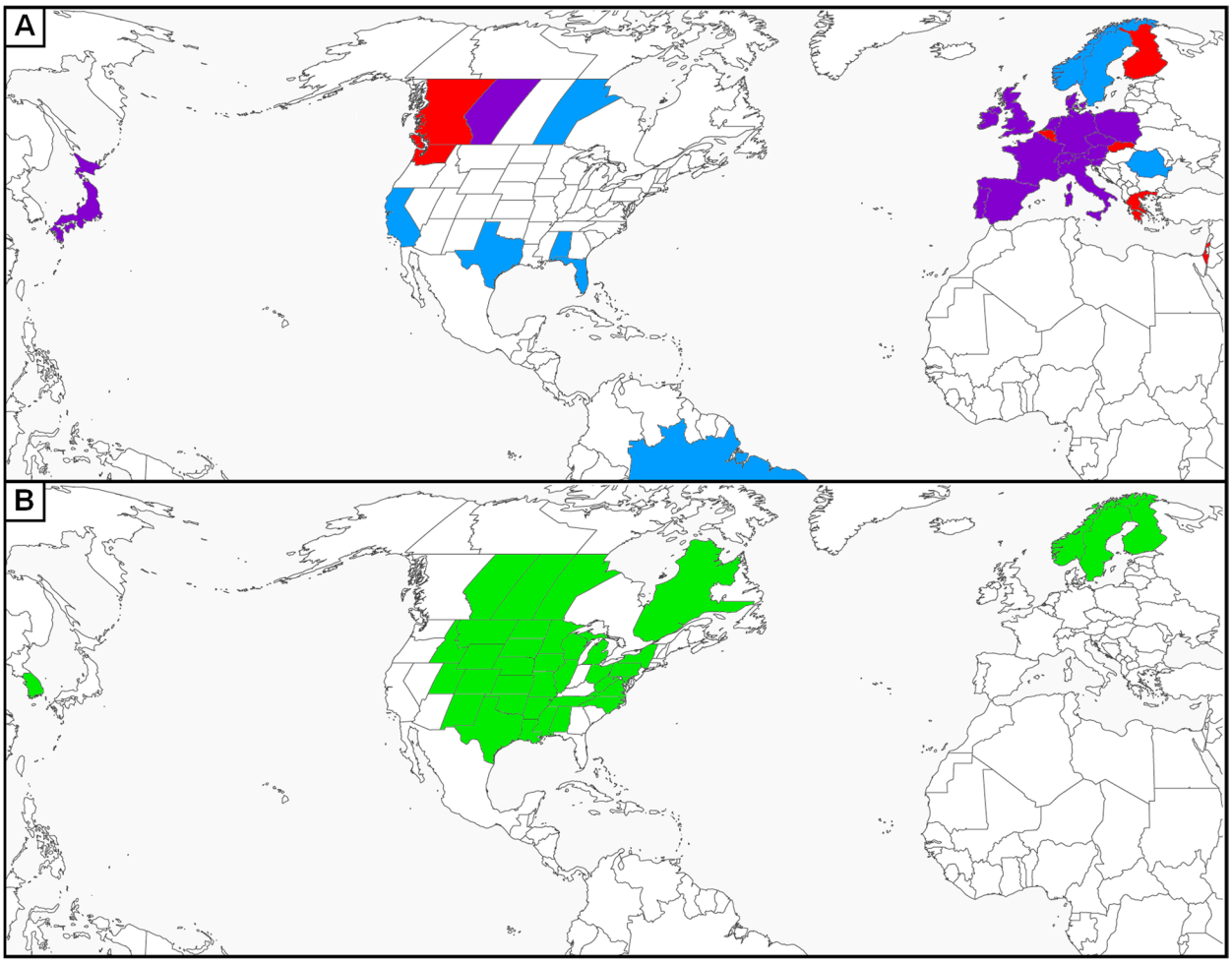

Fig. 1 Geographic extent of (**A**) classical and atypical BSE and (**B**) CWD. **A** States, provinces, and countries reporting cases of classical BSE (c-BSE) are shown in red. Note that the single case of c-BSE reported in Washington state in the U.S. was imported from Canada (United States Department of Agriculture 2004). Locations reporting cases of atypical BSE are shown in blue, while those reporting a mixture of both classical and atypical forms of BSE are shown in purple. In 2014, Romania reported two cases of BSE – one was confirmed as an atypical subtype, while the second was seemingly lost to follow up (European Food Safety Authority 2021; ProMED-Mail 2016). **B** States, provinces, and countries reporting cases of CWD in either farmed or free-ranging cervids are shown in green (Richards 2023)

evidence points to host factors playing a key role in direct animal to animal transmission, while other experimental findings suggest the prion agent itself also modulates horizontal transmissibility. Ultimately, both host and agent likely contribute important pieces of the transmission puzzle.

Apart from transmission routes, there are other important distinctions between these two diseases, including pathogenesis, tissue distribution of the prion agent (Fig. 2), and the potential for distinct prion "strains." (Greenlee and Greenlee 2015) Perhaps the most important distinction between the two is their zoonotic potential; whereas CWD has not demonstrably resulted in cases of human prion disease, upwards of 230 cases of variant Creutzfeldt-Jakob disease (vCJD) have been attributed to the consumption of BSE-contaminated beef or beef products (Houston and Andreoletti 2019). Some of the distinctions identified between the two diseases may ultimately be the root cause of their transmissibility differences, though it remains unknown whether there may be cases of CWD that are not transmissible, or whether a highly transmissible form of BSE may someday be identified.

This review will focus on what is known about CWD and BSE (primarily the classic form, with atypical forms noted where appropriate) and the agents responsible for them – representing the prototype of either a horizontally transmissible or non-transmissible prion disorder, respectively. Along with clinical symptoms, diagnosis, and management, key differences in the pathogenesis, transmission, and both geographic and host range of CWD and BSE will be presented – including data from cross-species transmission studies (e.g., cattle inoculated with CWD and cervids inoculated with c-BSE) that may provide insight into the specific roles of the host and the agent in the disease process; importantly, these studies inform on the risk of the possibility for the development of a horizontally transmissible prion disease in human populations. Finally, we will present future research directions, noting additional studies that may shed further light on the nature of highly transmissible prion diseases.

Bovine spongiform encephalopathy

Bovine spongiform encephalopathy (BSE or "mad cow disease") was the most economically important prion disease in veterinary medicine in the late 20th and early twenty-first centuries; in the 1990s, it was linked to a newly reported variant of Creutzfeldt-Jakob disease (vCJD) in humans (Collinge et al. 1996; Bruce et al. 1997; Ironside et al. 1996). Clinical symptoms of BSE are typical of those found in other prion diseases, including

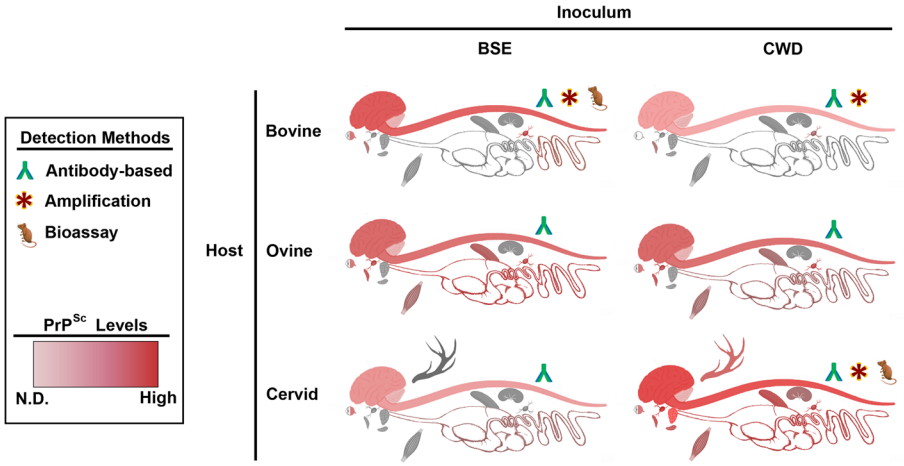

Fig. 2 Central and peripheral prion accumulation in different species infected with classical BSE or CWD. Generally, peripheral accumulation of either classical bovine spongiform encephalopathy (c-BSE) (Balkema-Buschmann et al. 2011; Franz et al. 2012; Ackermann et al. 2017; Ackermann et al. 2021; Iwata et al. 2006; Buschmann and Groschup 2005) or chronic wasting disease (CWD) (Hamir et al. 2007; Hamir et al. 2011b; Hamir et al. 2006c; Hamir et al. 2005; Haley et al. 2016c) prions is limited in cattle – with accumulation observed in the retina, tonsils, and mesenteric lymph nodes in animals inoculated with either c-BSE or CWD, along with the distal gastrointestinal tract of cattle infected with c-BSE. Sheep show a mixed distribution when infected with either c-BSE (Keulen et al. 2008; Lezmi et al. 2006; Jeffrey et al. 2001) or CWD (Cassmann et al. 2021a; Cassmann et al. 2021b; Hamir et al. 2006b), including deposits in tonsil, spleen, gastrointestinal tract, retina, and both retropharyngeal and mesenteric lymph nodes. Deer inoculated with BSE show a peripheral distribution similar to that of cattle with c-BSE, though lacking deposition in the tonsils and mesenteric lymph nodes occasionally seen in cattle with c-BSE (Dagleish et al. 2008; Martin et al. 2009). Most cervid species naturally infected with CWD have widespread peripheral prion accumulation across excretory organs, lymphoid tissues, as well as muscle and antler velvet (Sigurdson et al. 2001; Haley et al. 2011; Angers et al. 2009; Angers et al. 2006; Wild et al. 2002; Spraker et al. 2002a). Detection methods, including conventional western blotting or IHC, amplification techniques, or bioassay, varied across studies and are indicated for each assessment where appropriate

weight loss and behavioral abnormalities (apprehension, depression, etc.). Progressive neurologic dysfunction is also present in advanced cases, with additional symptoms including hyperesthesia, hyperreflexia, muscle tremors, ataxia, pruritis and reduced autonomic function (e.g., reduced rumination and bradycardia) (Wells et al. 1987). Incoordination and inability to rise are important discriminators that are often used to restrict cattle from conventional slaughterhouse processing. Both classical and atypical (spontaneous) subtypes of BSE have been identified, as noted above, and may be differentiated by western blotting analysis of molecular weight and glycosylation profiles (Fig. 3) as well as select amplification-based assays (Orru et al. 2015; Porcario et al. 2011).

Classical BSE first emerged as a newly recognized TSE in England in 1986, showing a rapid increase in incidence over the next few years (Smith and Bradley 2003; Wells et al. 1987). Soon after, novel TSE diseases were reported in captive zoo ungulates and felids, as well as domestic cats in the United Kingdom (Aldhous 1990; Kirkwood and Cunningham 1994). Cases of vCJD, "spillover events" arising from human consumption of BSE-contaminated beef, sausage, and other cattle byproducts, were first identified in 1996 and were distinguished from sporadic CJD cases based on age of onset and other clinical findings (Will et al. 1996).

Soon after the detection of c-BSE in the United Kingdom, reports of additional infected cattle began appearing in countries across Europe. These cases were ultimately linked to the export of infected animals, BSE-contaminated meat and bone meal (MBM), and possibly other contaminated animal products from the UK to the European continent. Since its initial descriptions, c-BSE has been reported in 26 countries, including cases in Europe, Israel, Japan, and North America, while atypical BSE cases have been reported in most European countries, North America, Brazil, and Japan (Fig. 1A) (European Food Safety Authority 2021).

Epidemiologic analyses strongly suggest that the primary mode of c-BSE transmission is through the ingestion of feed containing BSE-contaminated MBM (Smith and Bradley 2003). Since a nearly global ban on the use of MBM in ruminant feed, the incidence of the c-BSE has decreased sharply, with just a handful of cases reported over the past decade. Since 2013, the majority of the roughly 30 cases of BSE reported worldwide have been of the atypical subtype (European Food Safety Authority 2021). There has been no definitive evidence of horizontal transmission of either classical or atypical forms of BSE (Ferguson et al. 1997). Young cattle appear to be more susceptible to infection with the classical form than adults, and there is an increased risk of c-BSE in calves born to c-BSE affected cows (Wilesmith et al. 1997). The mechanism responsible for this increased risk in calves is unknown, however the possibility of factors such as milk replacer (Tsutsui et al. 2008), or less likely maternal transmission in utero or post-parturition (Hoinville et al. 1995), have not been completely ruled out. There is no strong evidence for environmental transmission of either subtype.

Wild ungulate and felid TSEs, as well as vCJD cases, suggest that c-BSE has an unusually wide susceptible

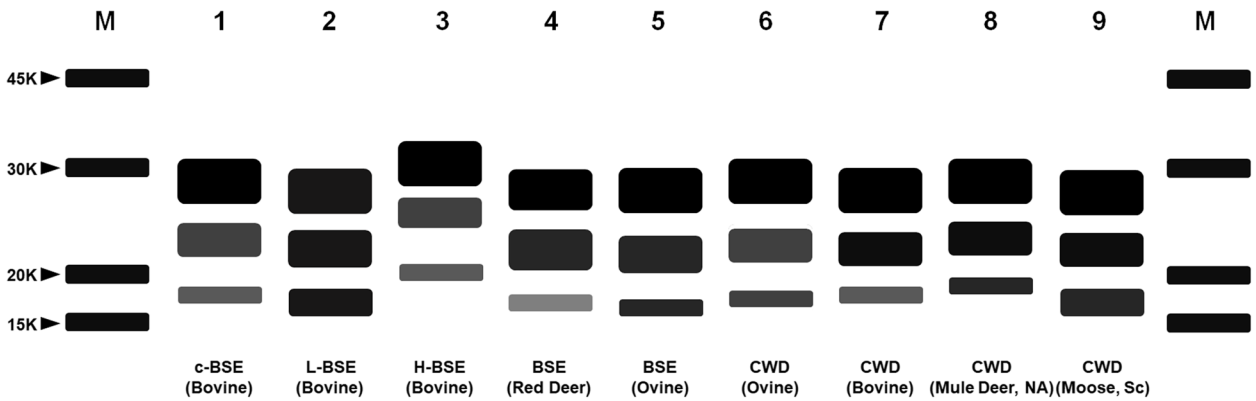

Fig. 3 Molecular characteristics of PrP^{res} in different species infected with either BSE or CWD. Differentiation of the various forms of bovine spongiform encephalopathy (BSE) – including classical (Lane 1) and both L- and H-type atypical BSE (Lanes 2 and 3, respectively) – is readily accomplished by comparing the relative molecular weights, weight range, and ratios of the di-, mono-, and unglycosylated PrP^{res} bands (Porcario et al. 2011). These profiles are distinct from BSE isolated in experimental studies of BSE in red deer (Lane 4) and sheep (Lane 5) (Martin et al. 2009). Isolates of chronic wasting disease (CWD) prions from experimentally infected sheep (lane 6) (Hamir et al. 2006b) and naturally infected mule deer from North America (NA, Lane 8) (Williams 2005) are quite similar, though distinct from isolates from both experimentally inoculated cattle (Lane 7) (Hamir et al. 2005) and a novel isolate from a naturally infected moose in Scandinavia (Sc, Lane 9) (Sun et al. 2023). Relative molecular weight and glycosylation profiles may vary subtly across brain regions and host Prnp genotypic background

host range, unlike other known TSEs. A handful of cases of c-BSE have also been reported in goats, likely through consumption of contaminated feed (Spiropoulos et al. 2011). Experimental infections with c-BSE have been reported in pigs, red deer, several non-human primates, and mice, although subsequent horizontal transmission between any of these species has not been demonstrated (Hedman et al. 2016; Dagleish et al. 2008; Piccardo et al. 2012; Herzog et al. 2004; Barlow and Middleton 1990). Studies of experimental c-BSE in sheep, described in detail below, have in contrast found some evidence of animal-to-animal transmission (Jeffrey et al. 2015). Because of its wide host range and particularly due to its association with vCJD, BSE has been considered a major threat to public health, forming the basis for national surveillance and eradication programs around the world.

Etiologic Agent

BSE, like other prion diseases, is caused by a misfolded protein, sometimes denoted as PrP^{BSE}. The normal bovine prion protein is 264 amino acids in length, with an unstructured N-terminal domain and a highly structured C-terminal domain, typical of other mammalian prion proteins. The bovine prion protein shares a relatively high sequence homology (86%) with the 253 amino acid human prion protein, particularly in the structured C-terminal domain. Several different subtypes, including the classic form and two atypical forms – L-type (sometimes referred to as bovine amyloidotic spongiform encephalopathy or BASE) and H-type, have been described (Porcario et al. 2011). Each subtype can be distinguished from one another by determining the molecular mass and the degree of glycosylation of PrP^{res} by western blot analysis (Fig. 3). Atypical L-type BSE is primarily characterized by a slightly lower molecular mass of the unglycosylated PrP^{res} band, compared to that of c-BSE PrP^{res}, while the H-type shows a higher molecular mass for the two glycosylated and single unglycosylated bands than those of c-BSE.

Most cases of atypical BSE have been detected through active national surveillance programs originally developed to detect cases of c-BSE, through targeted sampling of sick and downer cattle as recommended by World Organization for Animal Health (WOAH; formerly OIE) (World Organization for Animal Health (OIE) 2022). In general, these atypical BSE cases do not have distinct clinical signs as described for c-BSE. Most importantly, the majority of atypical BSE cases occur as single cases in a herd and are reported in cattle over 10 years of age. During active surveillance for BSE in France from 2001–2007, the estimated incidence of atypical BSE was 0.41 and 0.35 cases per million for H- and L-type forms, respectively (Biacabe et al. 2008). Case incidence in animals over 8 years of age was higher, with 1.9 and 1.7 cases per million for H- and L-type forms, respectively. This frequency is similar to that of sporadic CJD in humans, which has a yearly incidence of roughly 1 case per million people – typically appearing in those between the ages of 55 and 75; this supports the hypothesis that these atypical BSE cases are sporadic and spontaneous in nature (Parobkova et al. 2020).

Disease origins

The initial source of infectious material responsible for the emergence of c-BSE in the United Kingdom may never be known. Several theories have been provided to explain its emergence, including: the feeding of MBM containing (i) scrapie-contaminated sheep offal to cattle, either unchanged or structurally modified following changes made to traditional rendering processes (Taylor 1989; Huor et al. 2019; Brown and Bradley 1998); (ii) BSE prions from a single spontaneous or genetic case of BSE (Phillips 2000; Capobianco et al. 2007); or (iii) from animal feed imported from the Indian subcontinent that had been contaminated with the remains of humans suffering from one of the various human prion diseases (Colchester and Colchester 2005). A significant increase in sheep populations throughout the UK, and a subsequent increase in scrapie incidence prior to the emergence of c-BSE supports a possible link between the two diseases (Collee and Bradley 1997). Cross-species transmission studies of sheep scrapie into cattle, however, found that microscopic pathology and western blot profiles were distinct from both scrapie in sheep and c-BSE – hinting that it may be unlikely that the BSE epidemic had arisen directly from a crossover of scrapie into cattle (Konold et al. 2015; Cutlip et al. 1997; Cutlip et al. 1994). Regardless of the source, epidemiologic evidence suggests that a change in the rendering process in England in the late 1970s may have allowed for the increased stability of misfolded prion proteins in MBM, allowing for increasing levels of cattle exposure and ultimately an epidemic that would not be curtailed until the practice of feeding MBM was halted in the UK in 1988 (Woodgate and Wilkinson 2021).

Pathogenesis

Following oral exposure, c-BSE PrP^{Sc} is first detected in lymphoid follicles of the distal ileum by approximately 6 months post-inoculation (PI) (Balkema-Buschmann et al. 2011). The agent may occasionally be recovered from tonsilar by ~ 10 months PI (Wells et al. 2005). By most accounts, c-BSE pathogenesis bypasses further lymphoid accumulation in the periphery, including excretory tissues, and although PrP^{Sc} has been detected in several peripheral tissues using experimental amplification

approaches (Franz et al. 2012; Ackermann et al. 2017; Ackermann et al. 2021), it has not been detected to any significant degree in retropharyngeal, mesenteric, popliteal lymph nodes, or the spleen of naturally infected cattle using conventional immunohistochemistry (IHC), western blotting, or transgenic mouse bioassay (Fig. 2) (Iwata et al. 2006; Buschmann and Groschup 2005). By 32 months PI, the agent appears in the CNS and dorsal root ganglia, followed by trigeminal ganglia at 36 months PI, likely transported via retrograde axonal transport in peripheral nerve endings innervating gastrointestinal targets. Once reaching the CNS, PrP^{Sc} progressively accumulates and is associated with degenerative CNS lesions that are generally similar to those reported in other prion diseases (Simmons et al. 2011; Okada et al. 2011). As with other TSEs, there is no specific immunologic response to the PrP^{Sc} agent (Aguzzi and Heikenwalder 2006).

Transmission

As noted above, the transmission of c-BSE is dependent on the consumption of BSE-contaminated feeds, e.g., rendered MBM derived from BSE-affected cattle. The lack of detectable PrP^{Sc} in broader peripheral lymphoid tissues and excretory organs likely correlates with the absence of horizontal c-BSE transmission, unlike what has been reported with CWD (Miller and Williams 2003). For that reason, a ban on the use of MBM in ruminant feed in the UK in 1988 essentially broke the transmission chain of c-BSE. A further ban on bovine offal in 1990, so called "specified risk material," including skull, CNS, eyes, tonsils, and the distal ileum of the small intestine, from animal and human food chains further precluded the transmission of c-BSE to ruminants and other species (Heim and Kihm 2003). Despite these measures, evidence suggested that new feedborne cases continued in European cattle (Hazards EPanel oB et al. 2017); for additional food security, an earlier ban on cattle offal in animal feed was extended to a total ban on the inclusion of mammalian proteins in feed produced for any farm animals. Cases of c-BSE are now rare, outnumbered 5-to-1 by sporadic cases of atypical BSE over the past decade (European Food Safety Authority 2021).

Zoonotic potential

Zoonotic transmission of c-BSE to humans has been well documented, though has fortunately been a relatively rare occurrence, primarily due to the existence of a "transmission barrier" which limits the cross-species transmission of many TSEs (Houston and Andreoletti 2019; Manson and Diack 2016). This barrier is thought to be a result of differences in the primary structure between infectious source and host prion proteins as well as in the tertiary structure of the misfolded PrP^{Sc} protein and the host PrP^{C}. Experiments have shown that the tertiary structure of the misfolded c-BSE prion in particular – or more appropriately its "cloud of conformations" as sometimes described (Collinge 2010), likely plays an important role in its ability to overcome transmission barriers in a wide range of species. Because of this transmission barrier, just 230 cases of vCJD have been reported worldwide so far – with the majority reported in UK residents with a history of consuming beef products – despite over 180,000 known cases of c-BSE reported in the UK alone (Alarcon et al. 2023). Variant CJD cases in humans peaked a decade after the BSE epidemic peak in the early 1990s, with an estimated incubation period of 15–20 years in most cases (Valleron et al. 2001). Interestingly, nearly all cases of vCJD in humans shared a common Prnp genotype – homozygosity for methionine at position 129 of the Prnp gene (M129). An alternate allele, carrying valine at position 129 (V129), was found to confer a very high level of protection against vCJD infection (Saba and Booth 2013).

Variant CJD prions have been found by various methods in a range of human peripheral tissues (Douet et al. 2017; Diack et al. 2017; Notari et al. 2010; Head et al. 2004), with minor overlap reported in distribution compared to the parent c-BSE strain (Franz et al. 2012). An early study using an enhanced immunoblot technique identified vCJD prions in the thymus, tonsil, spleen, adrenal gland, and rectum of several affected human patients (Wadsworth et al. 2001). A separate study employing serial protein misfolding cyclic amplification (sPMCA), an in vitro prion misfolding amplification assay, identified amplifiable prions in a number of additional peripheral tissues, including exocrine organs like the salivary gland and kidney (Douet et al. 2017). These findings contrast the general restriction of prions to the CNS as described in cases of sporadic and familial human prion diseases (Head et al. 2004), though more sensitive amplification studies have not yet been performed for the other forms of CJD. A small number of cases have also arisen through blood transfusions from vCJD-infected donors (Ironside 2006), with several additional studies finding amplifiable prions in blood components from both clinical and preclinical vCJD cases (Concha-Marambio et al. 2020). These findings suggest that the pathogenesis of vCJD differs from not just c-BSE, but other forms of CJD as well, suggesting that the agent itself may have an important role in shaping prion disease pathogenesis. Further understanding of the roles of the host and agent in this process requires additional research, outlined in later sections.

Prevention and management

The prevention and management of c-BSE relies entirely on eliminating MBM and other specified risk materials

from cattle feed. While there have been no mutations within the cattle Prnp gene found to confer resistance to c-BSE, several insertion/deletion polymorphisms in the promoter region controlling Prnp expression levels have been associated with increased disease susceptibility and higher risk of atypical BSE (Clawson et al. 2008). Additionally, an E211K (glutamic acid, E, to lysine, K) polymorphism at position 211 within the bovine Prnp gene may be associated with H-type BSE, and may be the basis for heritable forms of BSE (Nicholson et al. 2008). This polymorphism is similar to an E200K polymorphism found in humans, which is thought to be associated with inherited CJD. Cattle that have had the Prnp gene "knocked-out" have been developed and shown completely resistant to c-BSE infection, though their utility, given the scarcity of c-BSE cases in the present day, is limited (Richt et al. 2007).

Chronic wasting disease

Chronic wasting disease was first identified in northern Colorado and southern Wyoming in the late 1960s and spread slowly and insidiously in this core area over several decades since its discovery (Williams and Young 1992). In the late 1990s and early 2000s, reports of CWD began appearing outside of its original core area, including cases in Wisconsin, Saskatchewan, and South Korea, in part through the movement of subclinically infected farmed deer and elk, and in part through natural wild cervid migration and the movement of infected carcasses (Haley and Hoover 2015). Over the past two decades, the endemic range of the disease has continued to expand across North America, with CWD having been reported in free-ranging or farmed deer in 31 U.S. states and four Canadian provinces (Richards 2023). In 2016, a novel CWD focus and strain was identified in reindeer in Norway, with additional cases and a second unique strain soon reported elsewhere in Scandinavia (Fig. 1B) (Tranulis et al. 2021). Absolute case numbers in North America are unknown, though likely extend well into the hundreds of thousands, while current reports in Scandinavia suggest a total of ~ 31 cases (Hazards et al. 2023).

Clinical symptoms of CWD are in line with those of other prion disorders in animals, with affected deer and elk showing behavioral changes including a reduced fear of humans or heightened aggression, along with variable neurologic defects, hair loss, and cachexia (Williams 2005). Increased salivation, bruxism, polydipsia, and polyuria are also common clinical findings in advanced cases. Clinical signs may never be apparent in free-ranging animals, as they are commonly removed from the herd in preclinical stages through predation, hunting, or vehicle collisions (Krumm et al. 2009; Krumm et al. 2005; Conner et al. 2000). In controlled settings, naturally and experimentally infected animals frequently die with signs of aspiration pneumonia, due to some combination of hypersalivation and dysphagia – a common cause of death in humans affected with various forms of CJD (Williams and Young 1992; Zerr et al. 2009). Incubation periods of CWD may range from 16 months to several years, and is strongly influenced by the Prnp genotype of the cervid host (Johnson et al. 2011; Hamir et al. 2006a).

A wide range of cervid species have been shown to be susceptible to CWD through oral transmission, including mule deer, white-tailed deer, North American elk, moose, caribou, red deer, sika deer, and muntjac. Non-cervid species have been found susceptible to CWD infection through experimental routes, e.g., intracranial inoculation, including some non-human primate species (Race et al. 2014), cattle (Hamir et al. 2007), swine (Moore et al. 2017), raccoon (Moore et al. 2019), domestic cats (Mathiason et al. 2013), ferrets (Bartz et al. 1998), and various rodent species (Heisey et al. 2010; Raymond et al. 2007). Fallow deer, interestingly, have been found to be resistant to natural routes of transmission, though are susceptible to intracranial inoculation (Hamir et al. 2011a). Collectively, the disease's continued expansion across North America and now Scandinavia in both farmed and free-ranging cervids, as well the natural and experimental susceptibility of cervid and non-cervid species, makes CWD the most important prion disease at this time.

Etiologic agent

The prion agent causing CWD is sometimes referred to as PrP^{CWD}. The normal prion protein in cervid species is 256 amino acids in length, with structural similarities to the bovine and human prion protein. The most common prion haplotype of white-tailed deer yields a protein that shares a 94% sequence homology with that of cattle, and an 89% sequence homology with the human prion protein, again primarily in the structured C-terminal end of the protein. While cases of CWD in North America are all considered infectious in origin and transmissible between susceptible cervid species, the appearance of CWD in Scandinavia – and the unique strains reported there – supports the potential for spontaneous origin of the disease (Pirisinu et al. 2018). There are thought to be a number of distinct CWD strains, based on differences in incubation periods and pathologic profiles in the natural host and laboratory species (Raymond et al. 2007; Perrott et al. 2012; Angers et al. 2010; Nonno et al. 2020; Sun et al. 2023; Otero et al. 2023). Based on western blotting profiles and studies in laboratory rodents, there is evidence that some isolates of CWD from Sweden and other parts of Scandinavia represent different strains than those present in North America (Fig. 3) (Pirisinu

et al. 2018; Nonno et al. 2020; Bian et al. 2021). Apart from some Scandinavian isolates, differentiating CWD strains biochemically in the natural host has not yet been possible. There is very little epidemiologic information available on the North American CWD strains, with no data available on differences in geographic distribution, species susceptibility, or incubation periods in the natural host.

Disease origin

Chronic wasting disease shares much in common with sheep scrapie, one of the earliest known prion disorders. There is some speculation that CWD may have arisen through direct or indirect transmission of scrapie from sheep, though its true origins are unknown. Research has shown that scrapie can be efficiently transmitted by intracranial inoculation to elk (Hamir et al. 2004) and via intracranial and oral routes to white-tailed deer (Greenlee et al. 2022; Greenlee et al. 2011), and similarly, chronic wasting disease can be transmitted to sheep via intracranial and oral routes, however with relatively low attack rates (Cassmann et al. 2021a; Cassmann et al. 2021b; Hamir et al. 2006b). Lesion profiles and molecular characteristics between the two diseases, including in cross-species transmission studies, are quite similar and support the scrapie-origin hypothesis for CWD (Greenlee et al. 2022; Cassmann et al. 2021a). No firm evidence is available, however, to confirm an historical link between scrapie and CWD in either North American or Scandinavian cervids, and it seems equally plausible that CWD arose spontaneously in deer species.

Pathogenesis

For most reported isolates, CWD prions initially appear in gastrointestinal-associated lymphoid tissues (GALT), similar to c-BSE, with additional appearance in retropharyngeal lymph node (RPLN), tonsil, and mesenteric lymph nodes along the distal GI tract by 6 weeks PI (Sigurdson et al. 1999; Sigurdson et al. 2001; Sigurdson et al. 2002). It should be noted that a recently described strain of CWD in a moose from Finland, however, failed to show any evidence of lymphotropism (Sun et al. 2023). Because of the early appearance of PrP^{Sc} in the RPLN, it is one of the preferred tissues for diagnosing CWD in deer postmortem (United States Department of Agriculture 2020). Sympathetic connections associated with the germinal centers in these lymphoid tissues may serve as a conduit for PrP^{Sc} to reach the central nervous system, with early accumulation and corresponding spongiform degeneration appearing in the dorsal motor nucleus of the vagus (Sigurdson 2008; Williams and Miller 2002; Balkema-Buschmann et al. 2019). The agent continues its advance to higher brain centers, with widespread prion accumulation and neurodegeneration coinciding with the onset of clinical disease (Williams 2005).

An incomplete picture remains concerning the timing of the appearance of PrP^{Sc} in peripheral tissues (Fig. 2), integral to horizontal transmission as described below. It is likely that the appearance in excretory tissues occurs concurrent with CNS ingress, with progressive accumulation over time in salivary glands, kidneys, etc. (Haley et al. 2011), similar to what has been reported for vCJD. The effect of Prnp genetics on the kinetics of PrP^{Sc} accumulation in cervid excretory tissues is likely significant. While it is often argued that animals with more prolonged incubation periods may exhibit a greater duration of environmental shedding (Plummer et al. 2017), the approximate onset of shedding in cervids with specific Prnp polymorphisms, discussed in additional detail below, is unknown.

Transmission

Much work has been done over the past two decades to characterize the routes of CWD transmission, relying on studies in both the natural host and transgenic mice, as well as experimental protein misfolded amplification techniques. Several natural routes are likely to be important, including horizontal transmission through excreta, vertical transmission either in utero or post-parturition, and of course through environmental contamination. Work still remains, however on characterizing the onset of shedding in infected animals and the persistence of CWD prions in various environments.

Saliva from animals in the clinical and preclinical phases of infection has been shown to carry significant levels of infectivity, approaching those which may be found in CNS tissues (Henderson et al. 2013). Other excreta, including feces and urine, have lower yet demonstrable levels of infectivity and detectable PrP^{Sc} (Tamguney et al. 2012). Using amplification and bioassay approaches, chronic wasting disease prions also have been found in various blood fractions, as has been reported for vCJD, as well as antler velvet, muscle tissue, and fat of CWD infected deer and elk (Fig. 2) (Mathiason et al. 2010; Angers et al. 2009; Angers et al. 2006; Race et al. 2009). Transmission of CWD from mother to offspring in utero seems likely based on separate reports of amplifiable prions in tissues from elk and deer fetuses (Selariu et al. 2015; Nalls et al. 2021), though the frequency of its occurrence is unknown. Thus far, there have been no reports of infectivity in colostrum or milk, though it is certainly possible based on the current

understanding of the disease and the potential transfer of scrapie and BSE via milk products (Tsutsui et al. 2008; Konold et al. 2013). There has been no evidence for the direct transmission of CWD through semen, however in vitro amplification approaches have identified low, potentially sub-infectious levels of CWD prions in semen from infected animals, and thus sexual transmission of CWD seems plausible as well (Kramm et al. 2019). Details on the timing of shedding in infected animals are not well understood, however infectious prions have been identified in various forms of excreta from preclinically infected deer and elk (Mathiason et al. 2009; Pulford et al. 2012). As noted above, it seems likely that Prnp genetic variations affect disease susceptibility and may shape the onset and duration of shedding in all forms of excreta and bodily fluids from affected cervids.

Apart from direct horizontal transmission, environmental contamination and transmission through infected carcasses and excreta is an equally important mechanism of disease spread and makes CWD incredibly difficult to manage – especially in free-ranging cervids (Miller et al. 2004). Using sensitive misfolded protein amplification techniques, CWD prions have been detected in water sources in endemic areas and from soil around mineral licks (Nichols et al. 2009; Plummer et al. 2018), and the agent has been shown to bind tightly to soil particles including clay and quartz – perhaps enhancing infectivity in the process (Johnson et al. 2007). CWD prions have even been experimentally recovered from plants cultivated in water containing CWD infected brain material and ticks collected from CWD-positive elk (Haley et al. 2021a; Pritzkow et al. 2015), although the precise role of plants and ticks in CWD transmission in nature is not yet known.

Zoonotic potential

To date, there have been no known cases of a human TSE linked to CWD exposure, despite the high sequence homology between cervid and human Prnp proteins. Several retrospective and prospective studies have been published without finding any evidence for zoonotic transmission, supporting the thought that the tertiary conformation of the infectious prions may be more influential than sequence homology for cross-species transmission (Olszowy et al. 2014; Belay et al. 2001). In vivo studies, using transgenic mice expressing various alleles of the human prion protein, and in vitro studies, examining amplification potential of CWD prions in human cellular prion substrate, have supported the hypothesis that, as it currently stands, the risk of zoonotic CWD transmission is negligible (Barria et al. 2018; Kong et al. 2005; Davenport et al. 2015; Hannaoui et al. 2022). It remains to be seen whether as-yet undiscovered CWD strains may have increased zoonotic risk.

Diagnosis

The diagnosis of CWD requires the examination of brainstem (at the level of the obex) and RPLN collected post-mortem (United States Department of Agriculture 2020). In white-tailed deer and mule deer, RPLN tissues have shown the greatest sensitivity, while in elk the obex is most often relied on for definitive diagnosis. Appropriate postmortem tissues in other cervids, including moose and reindeer, may vary, though generally both obex and RPLN are preferred (Benestad and Telling 2018). Samples are typically screened first via ELISA, with positive results subsequently confirmed by IHC (Hibler et al. 2003; Haley and Richt 2017). Because the sensitivity of both assays is imperfect, negative results are often simply reported as "not detected."

Antemortem testing approaches, using recto-anal mucosa-associated lymphoid tissue (RAMALT) or tonsil biopsies for example, are well documented though to date have not been approved for regulatory testing (Haley et al. 2020; Wild et al. 2002). The prion load, and therefore diagnostic sensitivities, of these tissues are lower than those of obex and RPLN tissues collected postmortem, typically 70–90% (Thomsen et al. 2012; Haley et al. 2016a; Haley et al. 2016b). Interestingly, antemortem test sensitivity may be significantly affected by the animal's Prnp genotype, with greater sensitivity reported in the highly susceptible genotypes described in the following section – most likely a result of the relatively rapid disease progression in these animals (Thomsen et al. 2012; Haley et al. 2016a; Haley et al. 2016b).

Experimental misfolded prion amplification techniques, including real time quaking-induced conversion (RT-QuIC) and sPMCA, have allowed for much greater sensitivity than conventional ELISA and IHC techniques in samples collected both antemortem and postmortem. They have additionally allowed for the evaluation of samples considered unsuitable using conventional methods, including saliva, nasal swabs, blood, urine, and feces (Henderson et al. 2013; Haley et al. 2016b; Henderson et al. 2017). Although these amplification assays have not yet been approved for regulatory testing, ongoing development and validation foreshadows their eventual approval for widescale use by regulatory agencies.

Prevention and management

Based on decades of research showing that selective breeding for scrapie resistant alleles in sheep has a significant impact in reducing scrapie prevalence (Hagenaars et al. 2010), an assumption might be made that a similar

approach would prove useful for managing CWD – at least in farmed cervids. Although several different alleles for the Prnp gene have been reported in various cervid species, none have yet been found to provide complete resistance to experimental CWD infection (Johnson et al. 2011; Hamir et al. 2006a; Robinson et al. 2012). There are several notable polymorphisms that have been identified in white-tailed deer, mule deer, or elk that have been associated with a lowered risk of CWD infection at the herd level as well as protracted disease incubation periods in individual animals, indicating some level of resistance may be possible (Haley et al. 2021b). These include the polymorphisms Q95H (glutamine, Q to histidine, H at position 95) and G96S (glycine, G to serine, S at position 96) alleles in white-tailed deer, a S225F (serine to phenylalanine, F at position 225) allele in mule deer, and a M132L (methionine, M to leucine, L at position 132) allele in elk. Animals heterozygous or homozygous for these mutations are underrepresented in naturally occurring cases of CWD, and when found to be infected are often in less advanced stages of the disease (Thomsen et al. 2012; Haley et al. 2016b; Haley et al. 2019). When experimentally infected, these animals have been found to have significantly longer incubation periods (Johnson et al. 2011). Notably, these alleles exhibit an additive effect with regard to differential susceptibility, whereby prevalence rates may be lower and disease course further prolonged in homozygous animals compared to those that are heterozygous. While these polymorphisms, and others in related cervid species, have not been found to confer complete resistance to CWD, ongoing genome-wide association studies may identify additional genes that may contribute to CWD resistance, and could ultimately lead to the selective breeding of highly CWD-resistant deer and elk (Seabury et al. 2022; Seabury et al. 2020). Any newly identified resistance-associated genes may also prove influential in managing other related prion diseases or protein misfolding disorders in general.

Because our current understanding of genetic resistance in cervids does not allow for unassailably effective CWD prevention and management, disease control must rely on more traditional approaches. In both farmed and wild herds, active surveillance is extremely important in identifying cases early and minimalizing both horizontal transmission and environmental contamination. Quarantine, trace outs, and depopulation is the most common approach for managing CWD in farmed herds, while herd reduction and dispersal strategies are often relied upon when managing CWD in wild cervid populations (USDA 2019; Conner et al. 2007; Geremia et al. 2015). The continued expansion of CWD in both farmed and wild cervid populations suggest that the development of more effective management approaches will be critical for controlling disease spread in the near future.

Cross-species transmissibility and pathogenesis studies

After reflecting on the similarities and stark disparities between c-BSE and CWD, an appropriate question may be "What are the host- and agent-derived factors involved, and which are the most influential, in the pathogenesis, horizontal transmissibility, and zoonotic potential of prion disorders?" One important approach in understanding the respective roles of host and agent in prion disease pathogenesis is cross-species transmissibility, i.e., investigating the pathogenesis of experimental CWD infection in cattle and sheep, or experimental BSE in cervid species and sheep. These experiments, performed over the past several decades, have provided valuable insight into the role of host and agent in TSE pathogenesis – insight which may ultimately help answer the question of whether a horizontally-transmissible prion disease, like CWD in cervids, could arise in human populations.

CWD in cattle

In experiments intended to estimate the natural susceptibility of domestic cattle to CWD, cattle were inoculated intracranially with isolates of CWD prions from several cervid sources, including mule deer, white-tailed deer, and elk (Hamir et al. 2007; Greenlee et al. 2012; Hamir et al. 2011b; Hamir et al. 2006c; Hamir et al. 2005). Although a small number of the cattle developed clinical signs suggestive of a prion infection, typically within ~ 2 years of exposure, there was an unexpected absence of overt microscopic pathology in any symptomatic animal. Molecular evidence of infection, however, was identified through immunostaining and experimental prion amplification, though limited to the central nervous system and, rarely, peripheral lymphatic tissues (Fig. 2) (Haley et al. 2016c). CNS deposition patterns and western blotting profiles of PrP^{Sc} were distinct from those reported for BSE, and yet dissimilar from those reported in CWD-positive animals as well (Fig. 3 and Table 1). No evidence for prion deposition in excretory tissues was found in any of the affected cattle – even in animals inoculated with second passage mule deer CWD previously passaged through cattle, unlike what might be expected with CWD in the natural cervid host. Subsequent experiments have shown that cattle are not susceptible to primary CWD exposure through the oral route, even following prolonged incubation times of over a decade (Williams et al. 2018). Cross-species transmission of scrapie to cattle have yielded similar findings, with limited peripheral accumulation and microscopic/

Table 1 Summary of PrP^{Sc} deposition in the CNS of different species infected with either BSE or CWD

Host and TSE strain	Telencephalon	Diencephalon	Cerebellum	Brainstem	Spinal cord	Deposition patterns	References
Bovine BSE	Mild to moderate staining in cortices and gray matter neuropil, including the hippocampus	Heavy staining	Moderate to heavy staining in cortical molecular layer	Heavy staining in the neuropil as well as occasional intranuclear staining in the DMNV and other nuclei	Mild to moderate deposition in gray matter and dorsal horns	Fine to coarse particulate, perineuronal, intraneuronal, intraglial, linear, or stellate depending on location with rare amyloid plaques	Simmons et al. 2011; Okada et al. 2011
Cervine BSE	Mild staining, typically gray matter	Mild staining in thalamic nuclei	Diffuse staining of the granular layer	Prominent in various nuclei of the medulla, including the DMNV	Diffuse staining throughout	Fine to coarse particulate deposits with occasional linear and stellate patterns	Dagleish et al. 2008; Martin et al. 2009
Ovine BSE	Limited staining in amygdala and putamen	Mild staining in the thalamus and hypothalamus	Mild to moderate staining in the granular cell layer	Prominent staining in the medulla and scattered nuclei, including the DMNV	Notable staining of the intermediolateral column	Fine to coarse particulate, granular, intraglial, stellate and linear throughout and occasional perineuronal staining in the telencephalon	Lezmi et al. 2006; Jeffrey et al. 2001
Ovine CWD	Variable depending on Prnp genotypes	Significant vacuolation in the thalamus	Staining across layers, throughout the gray and white matter	Most pronounced staining in medulla, primarily gray matter	Diffuse staining throughout	Intraneuronal, perineuronal, intraglial, coarse particulate ± stellate patterns	Cassmann et al. 2021a; Cassmann et al. 2021b; Hamir et al. 2006b
Bovine CWD	Mild to moderate staining, heaviest in the hippocampus; predominantly gray matter	Limited staining	White matter staining with sparse foci in granular layer	Mild to moderate staining, heaviest in the medulla and midbrain	Occasional staining in the white matter	Multifocal and distinct aggregates, scattered particulate or granular staining, and occasional small plaques. No perineural or perivascular staining observed	Hamir et al. 2007; Greenlee et al. 2012; Hamir et al. 2011b; Hamir et al. 2006c; Hamir et al. 2005
Cervine CWD	Heavy, diffuse staining	Heavy, diffuse staining	Moderate to heavy deposition in molecular, Purkinje, and granular cell layers	Diffuse staining, heaviest in the DMNV	Mild staining in the gray matter	Florid amyloid plaques common, depending on species and genotype, as well as perineural and perivascular deposits	(Spraker et al. 2002a; Spraker et al. 2002b

Descriptions of degree and distribution of PrPSc deposition of either classical bovine spongiform encephalopathy (BSE) or chronic wasting disease (CWD) prions, based on immunohistochemistry staining, are general in nature, and in most cases can vary based on disease stage and host Prnp background

molecular pathology distinct from both BSE and scrapie (Konold et al. 2015; Cutlip et al. 1997; Cutlip et al. 1994; Konold et al. 2006). Taken together, these findings suggest that it is the bovine host that dictates the pathogenesis of experimental CWD infection, making it unlikely that a transmissible form of CWD might develop in cattle. Neuropathology and molecular features of the misfolded prion, in contrast, may be influenced by both the initiating prion agent and the host.

BSE in cervids

In converse experiments, intended to assess the susceptibility of cervids to BSE, red deer (*Cervus elaphus*) were inoculated either orally or intracranially with cattle-derived c-BSE (Dagleish et al. 2008; Martin et al. 2009). In these experiments, red deer were highly susceptible to intracranial inoculation, and marginally susceptible to oral exposure. Incubation periods in symptomatic animals ranged from 26–42 months for intracranially inoculated animals, with just one of six orally exposed red deer developing clinical signs 57 months. Deposition patterns of PrP^{Sc} in red deer were distinct from those of CWD and more closely resembled those of bovine c-BSE, i.e., readily distinguished from CWD (Table 1). Western blotting and lesion profiles were also distinct from both c-BSE and CWD (Fig. 3). As was described above for CWD in cattle, no evidence for peripheralization of BSE prions in red deer was identified – in either intracranially or orally inoculated animals (Fig. 2). Secondary passage experiments and prion amplification approaches for peripheral tissues were not performed and may have provided additional insight into BSE pathogenesis in deer species. When considering these experimental findings, one might conclude that the agent itself is the primary force driving disease pathogenesis and transmissibility. This is very different from the observations reported above for experimental CWD infection in cattle.

BSE in sheep

Several studies over the past two decades have sought to understand the susceptibility of sheep to BSE (Konold et al. 2006; Keulen et al. 2008; Ronzon et al. 2006; Lezmi et al. 2006; Jeffrey et al. 2001). These studies generally found attack rates and incubation periods that followed patterns of sheep susceptibility to classical scrapie across varied sheep Prnp genotypes. Both central and peripheral PrP^{Sc} accumulation, including lymphoid and muscular tissues, was similar to that reported for classical scrapie, albeit measurably lower based on ELISA testing (Fig. 2). Western blot profiles and lesion profiles of sheep BSE prions were similar to those of some strains of classical sheep scrapie (Fig. 3 and Table 1). These findings would suggest the potential for some level of horizontal transmission of BSE in sheep; interestingly, low rates of transmission, particularly vertical transmission, were in fact reported in an intensively managed flock of sheep with experimental BSE infections (Jeffrey et al. 2015). The authors of that study noted that transmission rates were low enough that it would be unlikely for sheep BSE to persist in the flock. These studies support the heightened role of the host in TSE pathogenesis and transmission.

CWD in sheep

Several studies have likewise been conducted to better understand the pathogenesis of CWD in sheep following either intracranial or oronasal inoculation (Cassmann et al. 2021a; Cassmann et al. 2021b; Hamir et al. 2006b; Hamir et al. 2006c). Although these studies did not thoroughly consider the peripheral distribution of prions, they do provide some insight into the susceptibility, pathogenesis, and molecular profile of sheep-passaged CWD. In an early study investigating the susceptibility of Suffolk sheep to intracranial inoculation with mule deer-origin CWD, a low attack rate was reported among sheep with mixed Prnp genetic backgrounds, with just 2 of 8 sheep developing either a clinical or pre-symptomatic infection with incubation periods ranging from 35–72 months (Cassmann et al. 2021a) Distribution of PrP^{Sc} in the CNS was similar to those reported in sheep scrapie, with one sheep showing additional prion accumulation in peripheral lymph nodes (Fig. 2 and Table 1). Western blot PrP^{res} profiles were similar, but not identical to that of the original mule deer inoculum (Fig. 3). A follow-up study on secondary intracranial passage study in sheep was recently published, which found a 100% attack rate across varied Prnp genotypes, with all sheep having both central nervous system and peripheral accumulation of prions in both lymphoid and muscular tissues (Cassmann et al. 2021b). Disease progression across Prnp backgrounds was similar to, though not entirely consistent with those reported with sheep scrapie, which the authors note may have been a result of prion strain selection during primary passage. Finally, a more recent study examining the oronasal susceptibility of sheep to white-tailed deer derived CWD prions found a low attack rate, with just 1 of 7 sheep developing an asymptomatic infection after a 60-month incubation period (Greenlee et al. 2022). The lone TSE-positive animal did show peripheral lymphoid accumulation, and the authors noted that they could not rule out the potential for environmental shedding by this animal. In summary, these findings suggest that although the CWD and scrapie agents are distinct, both have the potential to disseminate to the periphery in a permissible sheep host – further implicating host factors as those most important in shaping prion disease pathogenesis and transmissibility.

Ultimately, the question of whether the host or the prion agent is more influential in prion pathogenesis and horizontal transmissibility remains incompletely answered. The preponderance of data from cross-species studies suggests that the host is the primary factor influencing pathogenesis and transmissibility, while both host and prion agent factors are capable of modulating lesion and western blotting profiles. Additional studies investigating c-BSE pathogenesis in species highly susceptible to CWD, e.g., white-tailed deer homozygous for the 96G Prnp allele, would allow a more complete understanding of the host's role in TSE transmissibility. Studies thus far seem to suggest that c-BSE would, in fact, show at least some peripheralization in white-tailed deer – as it had in sheep (Jeffrey et al. 2015) – with similarly low levels of transmissibility. By extension, these findings suggest that, based on findings of vCJD prion peripheralization, there is the distinct possibility that a horizontally transmissible prion disease could arise in humans following transmission of different animal prion agents such as CWD. With the sharp decline in BSE cases worldwide, and so far little evidence that CWD has any zoonotic potential, the risk of such a scenario appears to be quite low, however scientific studies to identify and assess the transmissibility of novel CWD, BSE, CJD and other emerging prion strains (e.g., camel prion strains (Babelhadj et al. 2018)) are essential to protect animal and public health and need to continue.

Future directions

To improve our understanding of the pathogenesis and transmission of prion diseases, several important studies should be considered going forward. More thorough investigations into the onset, duration, and sites of shedding of CWD prions in cervid species – especially those with newly identified strains or rare and arguably less susceptible Prnp alleles – will provide important information on how and when deer and related cervid species begin shedding infectious prions, and whether alternate Prnp genotypes or strains have any effects on shedding kinetics. To better appreciate the respective roles of host and agent in TSE transmissibility, more in-depth studies of BSE transmission to cervids, including prion amplification-based evaluations of available tissues from past studies in red deer, and new studies examining the susceptibility and pathogenesis of BSE in white-tailed deer are necessary; prion amplification-based studies targeting peripheral tissues from patients with various forms of CJD would also shed more light on this subject. Lastly, a better understanding of the zoonotic transmissibility of novel prion strains – including newly identified strains in moose, those isolated from cervids with rare Prnp alleles, the recently described camel prion disease, or cross-species experiments, would inform health professionals on the ongoing risk associated with consumption of and contact with TSE-affected cervids and other species.

Conclusions

Although BSE and CWD are both prion diseases of ruminants, there are significant differences in their pathogenesis, transmission routes, and zoonotic potential. These inherent differences raise questions about how a misfolded protein can behave so differently in different mammalian hosts; questions that are partially explored in experimental cross-species transmission studies that have provided us with a glimpse of the roles of the host and the prion agent in prion pathogenesis and transmissibility. Future studies expanding on these cross-species experiments and focusing in more detail on human prion disorders and newly emerging prion strains will allow a better understanding of the host versus agent role and, perhaps more importantly, will be able to determine the risk for a horizontally transmissible prion disease to arise in human populations.

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