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New Frontiers in Animal Prion Diseases

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Abstract

The transmissible spongiform encephalopathies are a group of fatal, progressive neurodegenerative disorders caused by the misfolding of prion proteins, leading to severe neuropathology and death. Since the description of scrapie in sheep several centuries ago, significant advancements have been made in understanding the spectrum of prion diseases, including bovine spongiform encephalopathy and Creutzfeldt–Jakob disease. Despite decades of research, critical gaps remain in our understanding of prion replication mechanisms, interspecies transmission, and the environmental persistence of prions. Advances in molecular imaging, including cryo-electron microscopy, have been instrumental in visualizing prion-associated aggregates in affected brain tissues, providing critical insights into their conformation and strain-specific structures. We explore the development of transmissible spongiform encephalopathy research in animals, major scientific breakthroughs, and the pressing need for innovative diagnostic and therapeutic approaches. Addressing these challenges is essential for controlling the spread of prion diseases, and reducing their impact on public health and agriculture.

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs) are a group of progressive, fatal neurodegenerative diseases caused by prions, misfolded, infectious proteins that can self-propagate by inducing normally folded proteins to adopt abnormal structures (1). TSEs are characterized by the accumulation of these misfolded prion proteins in the brain, leading to irreversible neuronal damage, a vacuolation in brain tissue, and death. These diseases affect both humans and animals, as shown in **Table 1** (2–4), and animal TSEs are of particular concern due to their zoonotic potential, as well as their impact on animal populations and public health.

The prominence of prion diseases has increased over time, with the earliest case of scrapie (a prion disease afflicting sheep) recorded in 1750 in South West England (5–7). Interestingly, the disease was likely to be recognized much earlier, with other names including "shaking," "rickets," and "rubbers" (8). Animal prion diseases became particularly prominent following the emergence of bovine spongiform encephalopathy (BSE) in the United Kingdom during the 1980s. This prompted the implementation of stringent containment policies, including feed testing and routine surveillance of animals to mitigate its spread (9). Despite these measures, and the subsequent reduction of BSE cases, the challenge of TSEs persists, as evidenced by sporadic cases of BSE reported in various countries and the identification of atypical BSE strains over the past 25 years, as well as the increasing spread of chronic wasting disease (CWD) affecting deer. With the initial identification of BSE occurring four decades ago, and the emergence of strains in new species (10), this review provides an overview of our current understanding of TSEs in animals, the ongoing importance of these disorders for animal and human health, and what the future holds for research.

THE PRION HYPOTHESIS AND PATHOGENESIS OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

The central event in TSE etiopathogenesis in all animals and humans is the misfolding of the normal cellular prion protein (PrP^{C}) into a pathogenic isoform (PrP^{Sc}). This involves a

Table 1 Comparative summary of prion diseases across species[a]

Transmissible spongiform encephalopathies	Primary host
Bovine spongiform encephalopathy	Cattle
Bovine spongiform encephalopathy–related disorders	Exotic ungulates, nyalas, zebu
Scrapie	Sheep, goats
Creutzfeldt–Jakob disease	Humans
Fatal familial insomnia	Humans
Kuru	Humans
Gerstmann–Sträussler–Scheinker syndrome	Humans
Variant Creutzfeldt–Jakob disease	Humans
Chronic wasting disease	Deer, elk
Feline spongiform encephalopathy	Domestic cats, cheetahs, ocelots, pumas, tigers
Transmissible mink encephalopathy	Domestic and wild mink
Camel prion disease	Dromedary camels

[a]This table summarizes the clinical signs of prion diseases in cattle (bovine spongiform encephalopathy), sheep (scrapie), deer (chronic wasting disease), and humans (variant and sporadic Creutzfeldt–Jakob disease). Common features include ataxia, behavioral changes, weight loss, and progressive neurological decline. Unique signs, like pruritus in scrapie and insomnia in variant Creutzfeldt–Jakob disease, highlight species-specific differences, underscoring both shared and distinct neuropathological characteristics of transmissible spongiform encephalopathies.

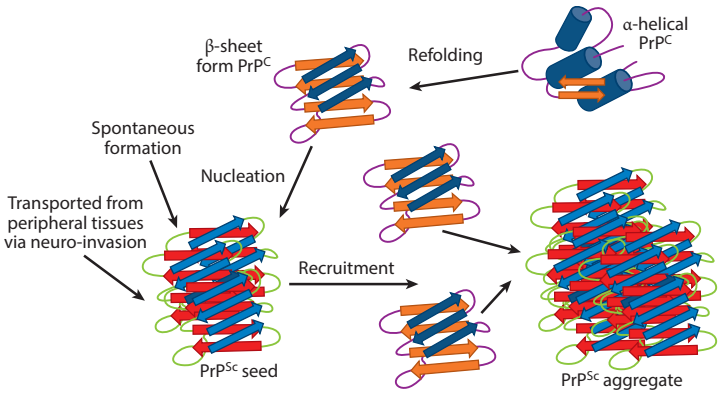

Figure 1

Prion hypothesis and propagation. The cellular prion protein (PrP^C) is highly conserved and is expressed in humans and different animal species within various tissues and biological systems. Levels of PrP^C are identified in the peripheral tissues, including skeletal muscle, kidney, and heart, with the highest levels identified in the central nervous system. Prion protein propagation refers to the process by which an abnormal, misfolded prion protein induces other, normally folded proteins to adopt its misfolded conformation, leading to the spread of the prion infection. The infectious form of the prion protein, often referred to as PrP^{Sc}, is structurally distinct from the normal cellular form (PrP^C) and contains a higher proportion of β-sheet structures. When PrP^{Sc} encounters PrP^C, it catalyzes the conversion of PrP^C into additional PrP^{Sc} molecules by templating the misfolding process. This process creates a chain reaction in which several PrP^C proteins are recruited and converted into PrP^{Sc}. As more PrP^{Sc} aggregates accumulate, they form amyloid fibrils that can damage cellular structures, particularly in the brain. This propagation mechanism is central to prion diseases, as the misfolded prions spread through tissues, leading to progressive neurodegeneration.

conformational shift from α-helical to β-sheet-rich structures, rendering the misfolded protein resistant to proteolysis and prone to aggregation. This aggregation-prone conformer disrupts cellular functions and leads to neurodegeneration. Uniquely, these protein aggregates can propagate between individual hosts and, in some circumstances, between species (11–14) (**Figure 1**).

PRION TRANSMISSION

Prion transmission in both humans and animals occurs primarily through exposure to infected tissue or environmental contamination, which can result from consumption of contaminated feed, direct contact with infected bodily fluids, or environmental sources. In animals, TSEs typically replicate first in peripheral lymphoid tissues, such as Peyer's patches in the gut, where they interact with the immune system before spreading to the central nervous system (CNS) via peripheral nerves (15–17). To date, no documented evidence indicates that prion diseases in humans can be transmitted through environmental exposure or through direct contact with infected individuals, including their bodily fluids, excreta, or contaminated surfaces.

In contrast, humans can develop iatrogenic prion diseases through direct exposure to the CNS, as observed in cases of Creutzfeldt–Jakob disease (CJD) linked to contaminated surgical instruments or dura mater grafts. Intriguingly, several scrapie outbreaks (in the United Kingdom in the 1930s and Italy in the 2000s) have been linked to the use of potentially infected vaccines, suggesting that iatrogenic transmission can occur in animals (18, 19). Upon reaching the CNS, PrP^{Sc} accumulates, resulting in the characteristic spongiform changes, neuronal loss, and gliosis that define the neuropathological progression of TSEs (20, 21).

Whereas animal TSEs such as scrapie in sheep or CWD in deer are acquired primarily through environmental exposure, with some cases apparently occurring sporadically, human prion diseases can be sporadic, genetic, or acquired. One notable example is the transmission of BSE from cattle to humans, causing variant CJD (vCJD) (22).

DISEASE VARIATION AND STRAIN IDENTIFICATION

Prion strains represent distinct conformational variants of PrP^{Sc}, each associated with unique biochemical properties, neuropathological profiles, and disease phenotypes (23). For instance, the structure of the prion fibrils from fatal familial insomnia (FFI) differs significantly from those of vCJD, consistent with their divergent clinical and neuropathological presentations (24).

In animals, including cattle, sheep, and deer, PrP^{C} is expressed in all organs but concentrated in neural tissue, where it plays a role in cellular signaling and maintenance of cell function (25). However, under certain conditions, typically when an animal is exposed to prion-contaminated material, PrP^{C} undergoes a conformational change into the misfolded PrP^{Sc}, forming aggregates that can deposit in the brain, leading to neurodegeneration (26). Different prion strains in animals, such as those causing scrapie in sheep or BSE in cattle, exhibit distinct molecular signatures, distinguished by altered protein conformations, glycosylation, patterns of pathology, clinical presentation, and species tropism. These differences contribute to varying incubation periods, disease progression, and susceptibility to transmission. In addition, animals like sheep have genetic polymorphisms in *PRNP*, the gene coding for PrP (e.g., the ARR allele) that influence susceptibility to TSEs like scrapie, with certain alleles resisting the disease. These genetic variations in animals highlight species-specific mechanisms for susceptibility (27, 28).

A defining feature of PrP^{Sc} is the inability to be completely digested by proteinase K like the normal cellular protein (29), which leads to cleavage at specific sites located at the N and C termini of the prion protein. This makes it a useful target for testing in Western blotting (WB), whereby the remaining protease-resistant fragment is then separated by SDS-PAGE and detected with prion-specific antibodies. Different prion strains yield distinct WB profiles based on the molecular mass of the di-, mono-, and unglycosylated glycoforms. For example, classical scrapie in sheep shows a characteristic triplet banding pattern on immunoblots, showing fairly equal distribution in the glycosylation bands. Similarly, BSE is associated with a defined glycoform ratio dominated by the diglycosylated band and an unglycosylated fragment around ~19 kDa. In contrast, H-type BSE (H-BSE) shows a higher–molecular mass unglycosylated band at ~21 kDa, and L-type BSE (L-BSE) presents with lower–molecular mass bands and reduced diglycosylated signal intensity. CWD shows a predominance of the diglycosylated band, similar to BSE; however, whereas CWD's unglycosylated band typically migrates around ~21 kDa, classical BSE (C-BSE) presents a slightly lower–molecular mass unglycosylated band around 19 kDa, highlighting subtle differences (30–32) (**Figure 2**).

Immunohistochemistry (IHC) uses antibodies against the undigested PrP^{Sc} protein, allowing for the visualization of amyloid deposits for detecting animal and human prion proteins such as BSE, scrapie in sheep and goats, and CWD in deer and elk. In C-BSE, PrP^{Sc} is typically found in the CNS, with minimal lymphoid and peripheral tissue expression. Classical scrapie, by contrast, often shows widespread PrP^{Sc} deposition in both the CNS and lymphoid tissues such as the tonsils and spleen. Except for a handful of CWD cases identified in moose and one in red deer in Nordic countries, CWD is marked by early and extensive PrP^{Sc} accumulation in lymphoid tissues, including lymph nodes and the gut-associated lymphoid tissue, before spreading to the brain (33, 34). These characteristics, in conjunction with amyloid deposition patterns, facilitate the differentiation of prion strains in animals, as depicted in **Figure 3** (35, 36). Overall, although the molecular

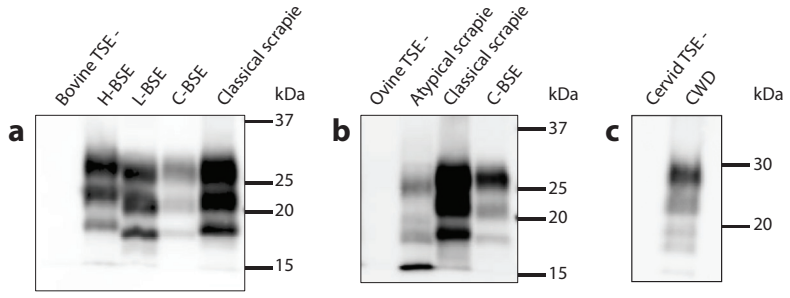

Figure 2

Western blot detection using SHA31 reveals distinct glycosylation patterns among prion strains. (*a*) C-BSE displays a characteristic three-band profile with a predominant diglycosylated band and an unglycosylated fragment migrating at ~19 kDa. H-BSE presents with a higher–molecular mass unglycosylated band (~21 kDa), whereas L-BSE exhibits a lower–molecular mass unglycosylated band (~17 kDa) and reduced diglycosylated signal intensity. (*b*) Classical scrapie shows relatively balanced intensities across all three glycoforms, whereas atypical scrapie is marked by a distinct, low–molecular mass fragment (~11–12 kDa), indicative of an alternative proteolytic cleavage site. (*c*) CWD demonstrates a C-BSE-like profile with subtle shifts in glycoform ratios. Abbreviations: BSE, bovine spongiform encephalopathy; C-BSE, classical BSE; CWD, chronic wasting disease; H-BSE, H-type BSE; L-BSE, L-type BSE; TSE, transmissible spongiform encephalopathies.

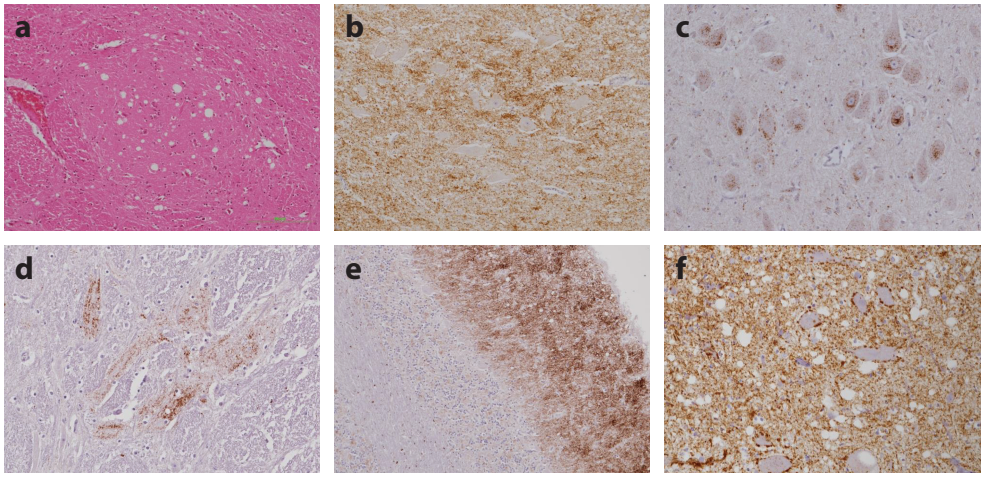

Figure 3

Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) images illustrating the distribution of disease-associated prion protein (PrP^{Sc}) in various transmissible spongiform encephalopathies (TSEs) in animals. (*a*) H&E-stained obex tissue from a case of L-type bovine spongiform encephalopathy (BSE), highlighting prominent vacuolation in the gray matter—a characteristic and pathognomonic lesion of all TSEs. (*b*) IHC staining of obex tissue from a classical BSE case, demonstrating widespread PrP^{Sc} accumulation; notably, H-type and L-type BSE obex tissues show a similar staining pattern in this region. (*c*) Classical scrapie in the obex, with diffuse and robust PrP^{Sc} deposition across the tissue. (*d*) Atypical scrapie with more localized PrP^{Sc} accumulation, restricted to the gray matter in the nucleus of the spinal tract of the trigeminal nerve. (*e*) The cerebellum from the same atypical scrapie case exhibits intense PrP^{Sc} staining, indicating this region as the most severely affected in the central nervous system. (*f*) The obex from the first confirmed case of chronic wasting disease in reindeer in Norway, showing positive PrP^{Sc} immunolabelling.

mechanisms of prion protein misfolding and aggregation are similar across species, species-specific variations in prion protein structure, strain diversity, and genetic background contribute to the distinct prion diseases observed in animals.

CLINICAL MANIFESTATIONS

Although the underlying mechanisms of prion-induced neurodegeneration are similar across species, the clinical manifestations can vary depending on the host and specific TSE strain involved. In animals, TSEs such as BSE, scrapie, and CWD are characterized primarily by behavioral changes, locomotor dysfunction, and sensory changes in some animals, such as hypersensitivity or pruritus. Humans affected by TSEs, including CJD, FFI, and kuru, experience cognitive decline, personality changes, and autonomic dysfunction. Despite these differences, both human and animal TSEs share key clinical features, such as progressive ataxia, changes in behavior or mental status, and eventual fatality. **Table 2** summarizes the major clinical signs observed in animal and human TSEs, highlighting their similarities and differences based on current scientific research (36–42).

SCRAPIE

Scrapie, the prototypic prion disease, has been recognized for at least 300 years, with reports from Germany and the United Kingdom dating back to at least the eighteenth century (43, 44). Described in both sheep and goats, scrapie is characterized by repetitive scraping behavior in response to intense pruritus, resulting in wool loss and skin damage (**Figure 4*a***), coupled with ataxia and dysphagia. The latter results in malnutrition and eventual death (45). Scrapie cases have been reported around the globe, and it is noted as being endemic in several European and

Table 2 Comparative clinical features of prion diseases in animals and humans[a]

Category	Animals	Humans	Similarities
Neurological signs	Ataxia, tremors, altered gait, incoordination	Progressive ataxia, tremors, muscle rigidity	Loss of coordination, tremors, balance issues
Behavioral changes	Increased aggression, nervousness, hyperexcitability	Personality changes, anxiety, depression	Altered behavior, mood disturbances
Cognitive impairment	Likely but difficult to test	Memory loss, confusion, dementia	Progressive neurological decline in advanced stages
Hyperesthesia	Heightened sensitivity to touch or sound	Pain sensitivity, abnormal sensory responses	Sensory disturbances
Weight loss and anorexia	Severe weight loss, decreased appetite, difficulty swallowing	Unintentional weight loss, difficulty swallowing	Muscle wasting, loss of appetite
Sleep disturbances	Disrupted sleep patterns	Insomnia, disrupted sleep patterns	Disturbed sleep in some cases
Autonomic dysfunction	Excessive salivation, difficulty swallowing	Dysautonomia, sweating abnormalities	Impaired autonomic functions
Fatal outcome	Progressive neurodegeneration	Rapid neurological decline	No cure, death inevitable

[a]This table outlines the principal clinical manifestations associated with prion diseases across animal and human hosts. Although cognitive impairment is documented predominantly in human cases, both groups exhibit notable convergence in neurological signs (e.g., ataxia, tremors), behavioral disturbances, autonomic dysfunction, and progressive neuromuscular degeneration. Despite interspecies variation in symptom expression and detection, the pathogenesis culminates universally in irreversible neurodegeneration and death.

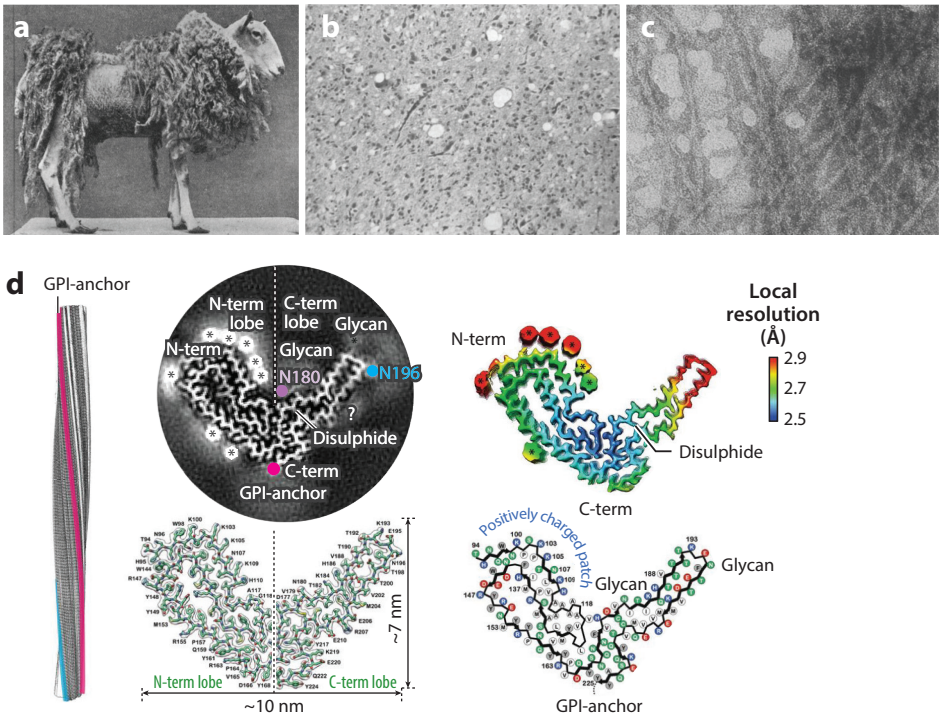

Figure 4

Scrapie from the animal to the molecular. (*a*) Sheep presenting with symptoms of scrapie, highlighting the sheep's disheveled appearance as a consequence of repetitive scraping in response to intense pruritus. Reproduced from Reference 127 (image in the public domain). (*b*) Spongiform degeneration in the brain of a sheep with end-stage scrapie. Reproduced with permission from Reference 128. (*c*) Amyloid fibrils isolated from the brain of a mouse infected with mouse-adapted scrapie strain 139A. Reproduced with permission from Reference 129. (*d*) The atomic resolution structure of prion protein fibrils isolated from mice infected with RML mouse-adapted scrapie prions. Adapted from Reference 130 (CC BY 4.0).

North American countries (46). The spongiform degeneration that defines the TSEs was first described in the brains of sheep with scrapie; likewise, scrapie-associated fibrils were first isolated from sheep brains (47) (**Figure 4*b*,*c***). Although the molecular cause of scrapie remained a mystery until recent decades, the transmissibility of the scrapie agent had been noted in the wild anecdotally in the 1700s and demonstrated experimentally by the 1930s (4). Importantly, scrapie could be transmitted to other Caprinae, including goats, as well as to a range of laboratory animals including mice and hamsters. In a natural setting, transmission is thought to occur predominantly via environmental exposure, although vertical transmission has been reported as well (48). Notably, and despite many centuries of dietary exposure, there is very little evidence that scrapie can be transmitted to humans. Importantly, the ability to model scrapie under controlled experimental settings was crucial in establishing the atypical nature of the scrapie agent, including resistance to formalin and radiation, and eventually resulted in the establishment of the prion hypothesis (7, 49, 50). More recently, experimentally derived scrapie prions have been characterized at an atomic resolution using cryogenic electron microscopy (CryoEM) (**Figure 4*d***).

Other important aspects of scrapie, with key implications for other prion diseases, are the natural occurrence of distinct strains and a genetic component to resistance. For the former, strains are distinguished by different clinical presentations, perhaps best exemplified by the drowsy and

scratching manifestations in goats (51). For the latter, some breeds of sheep are completely resistant to scrapie, a resistance that is now recognized to derive predominantly from variation in the ovine *PRNP* gene on chromosome 13 (52, 53).

CHRONIC WASTING DISEASE

CWD is a prion-mediated neurodegenerative disorder that affects members of the Cervidae family, including white-tailed deer, mule deer, red deer, elk, moose, and reindeer (54). The disease follows the general pathogenesis of abnormal prion protein accumulation in the CNS, particularly in the brain and spinal cord, leading to progressive neuronal degeneration, spongiform encephalopathy, and ultimately death. Since its initial discovery in captive mule deer in Colorado in 1967 and formal characterization in the 1980s (55), CWD has been detected in various parts of North America, Scandinavia, and South Korea, causing significant ecological concerns, especially regarding the potential impact on cervid populations and ecosystem integrity (56).

Clinically, CWD is characterized by signs such as severe weight loss (wasting), behavioral changes (e.g., loss of fear of humans), drooling, difficulty moving, and tremors, which are indicative of the neurodegenerative process. The prions responsible for CWD are highly infectious and can be transmitted through direct contact between infected and susceptible animals, as well as indirectly through contaminated environments (e.g., via saliva, feces, urine, and carcasses) (57). The possibility of transmission to humans has been the subject of intensive research; however, to date, there is no conclusive evidence that CWD can produce a disease in humans (58–60). Despite this, surveillance efforts continue, given the disease's persistence and expanding geographic range, now reported in more than 30 US states, several Canadian provinces, and parts of Europe (Finland, Norway, and Sweden) and Asia (South Korea) (61, 62).

Research into CWD's pathogenesis has revealed that environmental contamination plays a significant role in its transmission. Prions can persist in the environment for extended periods, further complicating efforts to control disease spread (63). Recent studies have also identified potential genetic factors that may influence CWD susceptibility in cervids, with certain genotypes appearing more resistant or more susceptible to prion accumulation (64). Additionally, researchers are exploring noninvasive diagnostic methods, such as fecal sampling and microbiome analysis, which could provide early detection tools for wildlife management programs (57). Despite these advancements, no effective vaccine or therapeutic intervention is available for CWD currently, and efforts are focused primarily on monitoring, managing, and mitigating its spread through population control, habitat management, and other regulatory measures (65).

As the disease continues to spread, it poses significant challenges to wildlife conservation, hunting industries, and ecosystems. The long-term ecological consequences of CWD on cervid populations and associated predators and scavengers remain poorly understood, and further research is critical to developing effective strategies for disease management and containment. Given the complexity of prion diseases and the absence of a cure, CWD remains a significant concern for wildlife health and its broader environmental implications.

TRANSMISSIBLE MINK ENCEPHALOPATHY

Transmissible mink encephalopathy (TME) is a prion disease that affects mink and is characterized by progressive neurodegeneration leading to vacuolation, neuronal loss, and gliosis in the CNS. TME was first identified in farmed mink in the United States in the 1990s (66), and although it remains relatively rare, it has raised significant concerns regarding prion transmission in agricultural settings. The primary route of transmission appears to be oral. Infected mink likely acquire the disease through the consumption of contaminated feed containing animal

by-products, particularly from other prion-infected species such as sheep (67) and, mainly, cattle, suggesting a possible cattle origin (68).

Clinically, TME manifests with varied neurological signs, including ataxia, tremors, excessive salivation, difficulty walking, and behavioral changes. Histopathological examination of affected brains reveals spongiform encephalopathy, characterized by vacuole formation in neuronal tissue, gliosis, and neuronal loss, like the brain pathology seen in BSE (69, 70).

TME's zoonotic potential remains unclear, because prion disease pathogenesis in mink and prion transmission mechanisms across species are still not understood fully. Some studies suggest a possible risk of transmission to larger ruminants, especially given the similarity in molecular features between TME and L-BSE (71–73). Although to date no evidence suggests that TME poses a significant threat to human health, further research is required to better understand TME epidemiology and the environmental factors influencing prion persistence (74).

BOVINE SPONGIFORM ENCEPHALOPATHY AND RELATED DISORDERS

BSE, colloquially known as mad cow disease, is believed to have originated in the United Kingdom in the late 1970s to early 1980s (10). It is classified into two distinct forms: C-BSE, which is epidemiologically linked to the development of vCJD, and atypical BSE, identified in 2004 (75, 76). The prevailing hypothesis is that c-BSE emerged through the recycling of animal by-products in cattle feed, particularly meat and bonemeal derived from scrapie-infected sheep, or spontaneously occurring prion diseases in cattle (77). The change in rendering methods during the 1970s, such as the use of lower temperatures and the discontinuation of solvent extraction, may have failed to fully inactivate prions, allowing them to survive in processed feed (78). As a result, the disease spread among herds until clinical cases began to appear, with the first officially confirmed BSE diagnosis reported in 1986. Once established, the disease led to epidemic in the United Kingdom and later in other countries, prompting widespread reforms in animal feed regulations and prion research [Regulation (EC) 999/2001].

Figure 5 presents the history of BSE from its first identification to the present (79–90). In contrast, atypical BSE occurs sporadically in aged cattle without any known association with contaminated feed and is considered a spontaneous prion disorder. This atypical form is further subclassified into H-type and L-type BSE, based on the biochemical and conformational properties of the abnormal PrP^{Sc}.

Although zoonotic transmission of C-BSE to humans has been well-documented, it has demonstrated the ability to transmit to several other species, including felines and other ungulates, both naturally and experimentally. During the BSE outbreak in the United Kingdom, numerous domestic cats and captive wild felids developed feline spongiform encephalopathy after presumably consuming contaminated meat products (91, 92). Interestingly, domestic dogs did not exhibit the disease, possibly due to species-specific differences in prion protein structure (93). Among ungulates, several captive wild bovids contracted spongiform encephalopathy, likely from contaminated feed. Experimental studies (94–97) have further shown that red deer can be susceptible to BSE through intracerebral inoculation, exhibiting clinical signs and neuropathology like CWD, although notably the resulting disease phenotypes are distinct and distinguishable (98). Additionally, although there are no natural cases, BSE has been experimentally transmitted to sheep via intracranial inoculation and ingestion, resulting in disease phenotypes resembling scrapie rather than BSE, highlighting the potential of interspecies transmission (99). These findings underscore the importance of stringent feed controls and ongoing surveillance to prevent interspecies transmission of BSE.

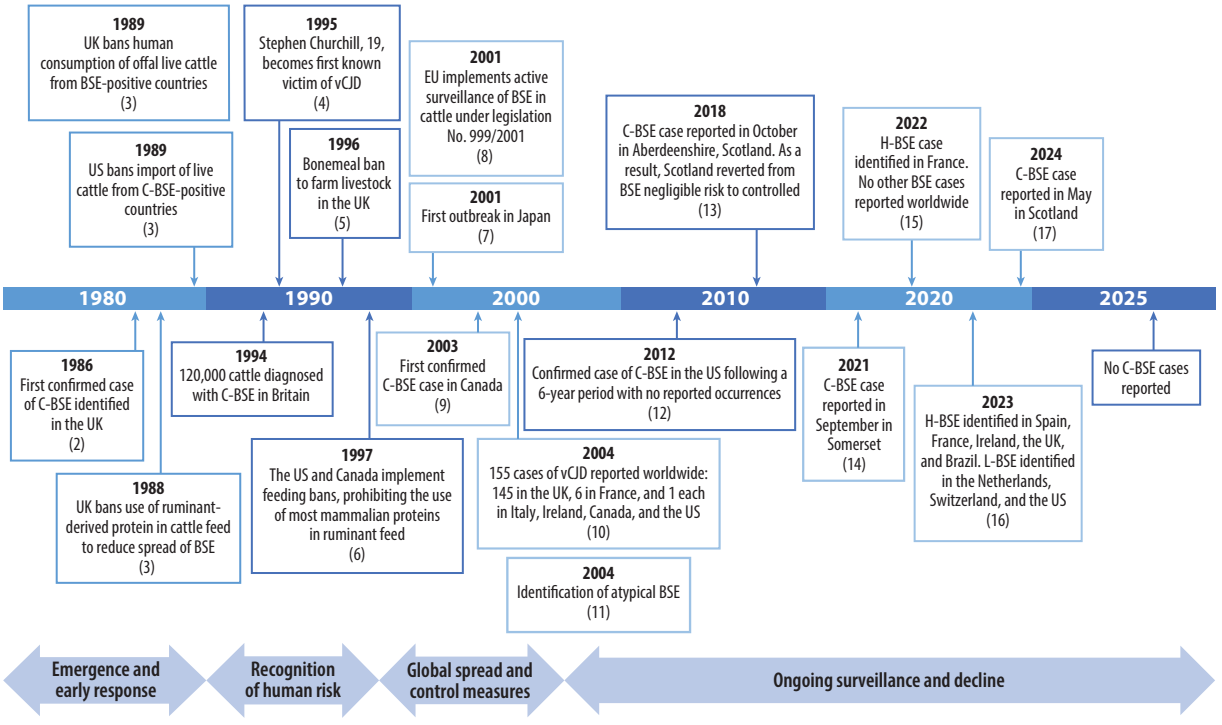

Figure 5

Timeline of key events in the history of BSE, 1986–2025. This figure highlights major milestones in the identification, regulation, and surveillance of BSE. It includes the first confirmed case in 1986, the emergence of vCJD in humans, global spread, implementation of control measures such as feed bans, and the discovery of atypical BSE strains. Recent years show ongoing but sporadic cases, underscoring the need for continued vigilance. Abbreviations: BSE, bovine spongiform encephalopathy; C-BSE, classical BSE; H-BSE, H-type BSE; L-BSE, L-type BSE; vCJD, variant Creutzfeldt–Jakob disease.

The emergence of L-type and H-type BSE, particularly in countries with robust BSE control measures, suggests that it may arise from a different prion strain or exposure pathway, possibly linked to natural prion propagation in cattle populations (100). H-type BSE is characterized by prion protein with a distinctive molecular profile, including an increased proportion of diglycosylated prion proteins (**Figure 2**). This type of BSE typically exhibits severe spongiform changes in the brain and is observed more frequently in cattle exposed to contaminated feed containing animal by-products. In contrast, L-type BSE is distinguished by a lower shift in the glycosylated molecular profile but is also thought to have histopathological changes similar to those of H- and C-BSE (**Figures 2** and **3**). L-type BSE is believed to have a higher potential for zoonotic transmission, with recent studies with nonhuman primates showing the potential for interspecies transmission (101). However, no cases of human TSE associated with L- or H-type BSE have been documented. It has been observed more commonly in countries with lower levels of BSE surveillance and control.

Although the exact mechanisms underlying the spontaneous emergence of H- and L-type BSE are not understood fully, genetic mutations or sporadic misfolding of normal prion proteins may lead to disease development. For instance, a specific mutation (E211K) in *PRNP* has been associated with H-type BSE and an L-type in some cases (102), yet the primary risk factor for atypical BSE remains its spontaneous emergence in older cattle. Because neither atypical BSE strain is

linked to prion transmission through contaminated feed, the differences in their molecular characteristics and ability to experimentally transmit to other species highlight their difference from C-BSE and their potential risks to both animal and human health.

HUMAN PRION DISEASES

TSEs in humans share key aspects of the etiopathogenesis of the animal prion disorders, with PrP^{C} converted into misfolded PrP^{Sc} aggregates, leading to neurodegeneration and disease. In humans, PrP^{Sc} can also exhibit resistance to protease digestion, and its aggregation may lead to the formation of amyloid plaques in brain tissue in some diseases, most notably Gerstmann–Sträussler–Scheinker syndrome. The molecular manifestations in humans are like those in animals, but differences in *PRNP* genotype, along with specific mutations or polymorphisms in the PrP gene, can directly cause familial prion disease. Additionally, human prion diseases often present with prominent clinical symptoms, such as rapid cognitive decline, ataxia, and myoclonus (103).

The human prion diseases include kuru and CJD, which exists in several forms: familial, iatrogenic, and sporadic CJD (sCJD), as well as vCJD. sCJD, the most common form, arises without any known genetic mutation or external exposure to prions, whereas familial CJD is inherited due to mutations in the prion protein gene (*PRNP*). vCJD, a subtype of acquired CJD, is linked to exposure to material from cows infected with BSE (104), whereas iatrogenic CJD is a rare form that can occur from exposure to contaminated medical equipment or surgical procedures, such as organ/tissue transplants or brain surgery. Other human prion diseases include FFI, which is caused by mutations in the *PRNP* gene and leads to a progressive loss of sleep and severe autonomic dysfunction, and Gerstmann–Sträussler–Scheinker syndrome, which is also associated with inherited mutations in *PRNP* and results in a range of neurological symptoms, including cerebellar ataxia and dementia (105).

In addition to the sporadic and familial forms of human prion disease, transmission between individuals has been documented in several human prion disorders. The prototypic transmissible human prion disease was kuru, associated with transumption among the Fore people of Papua New Guinea (106). This has been largely eradicated but remains an important historical example of epidemic prion transmission. Human prions, which are highly resistant to conventional sterilization methods, have been reported to be transmitted through the use of contaminated cadaveric tissue and through iatrogenic transmission via contaminated medical instruments (107).

FRONTIERS OF PRION RESEARCH

Recent advances in modern technologies have significantly enhanced our understanding of prion diseases. New technologies and approaches, such as genome-wide association studies (GWAS), advanced imaging techniques, and novel sensitivity assays, have facilitated sophisticated investigations into the molecular mechanisms of prion diseases. These tools permit researchers to analyze genetic variations, pinpoint biomarkers, and examine disease progression at new levels of precision. Moreover, the development of more sensitive diagnostic assays is improving our ability to detect prions in biological samples, even at early stages of disease. The application of these technologies is not only expanding the scope of prion research but also identifying therapeutic targets, early detection, and potential interventions to develop how prion diseases are studied, diagnosed, and ultimately managed.

Research into prion diseases over the past several decades has focused on understanding the molecular mechanisms that underlie prion propagation and pathogenesis. A recent study (108) provided new insights into how prions spread within the brain, revealing that prion proteins

nucleated protein self-assembly in a manner similar to other neurodegenerative diseases like Alzheimer's. This study highlighted the role of synaptic vesicles in prion internalization and transmission, suggesting potential targets for therapeutic intervention.

REAL-TIME QUAKING-INDUCED CONVERSION

Advances in diagnostic techniques have led to the development of more sensitive assays such as real-time quaking-induced conversion (RT-QuIC), which allows for the detection of misfolded prions in cerebrospinal fluid and other biological samples at earlier stages of disease. Compared to its predecessor, protein misfolding cyclic amplification, which used a similar principle by incubating potential prion-infected samples in healthy brain tissues, RT-QuIC uses recombinant prion proteins that mimic cellular PrP but standardizes the technique, making it repeatable and using less biological material. This can be advantageous for premortem testing in the early stages, when symptoms may not yet be apparent. The assay is valuable due to its high sensitivity, low cost, and ability to work with minimal sample volumes, making it a promising tool for both clinical diagnosis and surveillance of prion diseases in both humans and animals. Moreover, RT-QuIC has potential applications in tracking disease progression and evaluating the efficacy of therapeutic interventions (109).

A recent study demonstrated the efficacy of RT-QuIC in detecting BSE-associated prions in subclinical cattle. Researchers observed that RT-QuIC identified prion seeding activity in brain regions such as the midbrain and thalamus, even when traditional diagnostic methods like IHC and WB failed to detect PrP^{Sc}. This finding underscores RT-QuIC's superior sensitivity in identifying prion presence during early or subclinical stages of BSE infection (110), with the possibility of detecting differences between atypical BSE and the classical strain.

Another recent study used RT-QuIC to detect prion positivity from patients with sCJD and non-prion diseases as controls. Positive signals were observed in both lobes, with the frontal lobe showing a higher seeding dose compared to the temporal lobe. These findings suggest that RT-QuIC is a valuable tool for detecting low levels of prion infectivity in human tissues, even when the prion protein concentrations are minimal (111).

By amplifying prion seeding activity in biological samples, RT-QuIC offers a faster, more reliable alternative to traditional diagnostic methods, such as IHC and WB. Its versatility and efficiency make it an essential tool in both clinical diagnostics and surveillance, with the potential to significantly improve early detection, monitoring, and control of prion diseases (112).

THE ATOMIC RESOLUTION STRUCTURE OF PRIONS

CryoEM is a powerful imaging technique used to study prion diseases by providing high-resolution, detailed images of prion-associated structures at the ultrastructural level (113). In prion research, CryoEM is particularly useful for visualizing the accumulation of misfolded prion proteins, such as PrP^{Sc}, within affected tissues. This technique can reveal distinct morphological features, such as amyloid fibrils and the characteristic plaques formed by prions in the brain. Electron microscopy also aids in identifying changes in cellular architecture caused by prion-induced damage, which is crucial for understanding disease progression. Although CryoEM is not typically used for routine clinical diagnosis due to its complexity and cost, it remains an important research tool for exploring the pathogenesis of prion diseases and investigating the molecular mechanisms underlying prion protein aggregation and spread (113).

Recent studies employing CryoEM have provided significant insights into the structural characteristics of prions associated with scrapie, a prion disease affecting sheep. In 2021, researchers used CryoEM to determine the near-atomic resolution structure of infectious prion

fibrils derived from the 263K strain of mouse-adapted scrapie. This study revealed that the fibrils consist of parallel, in-register intermolecular β-sheets, with each monomer contributing a rung to the fibril core. Notably, certain sugars and glycosylphosphatidylinositol anchors project outward from the fibril core, suggesting their potential role in prion propagation and interaction with cellular membranes (114). Subsequent work has enabled direct comparison of different mouse-adapted scrapie strains (115), CWD prions (116), and human prions (117). These studies could provide critical insights into the structural basis for prion propagation and clarify the distinctions between different strains and types of prions. As a standout example, this technology provides the possibility of testing whether the prions found in the brains of cows with BSE and humans with vCJD share the same structural characteristics at an atomic resolution.

The use of electron microscopy to distinguish between different types of BSE has significant potential to influence policy changes related to prion disease surveillance and control. L-BSE, previously purified, has shown two structural classes of amyloid fibrils (118), which, if performed with C-BSE and H-BSE, could lead to more accurate and rapid identification of prion diseases in cattle populations, especially in asymptomatic or subclinical cases.

Additionally, recognizing the unique characteristics of each BSE type could allow for more targeted control measures. For example, if certain BSE variants exhibit different transmission patterns or infectious profiles, specific containment or culling strategies could be developed to prevent the spread of strains. In the long term, these advancements in prion diagnostics could lead to more effective policies for preventing prion disease outbreaks and safeguarding both human and animal health.

IMPLICATIONS FOR OTHER PROTEIN AGGREGATION DISORDERS

Prion diseases such as CJD have provided valuable insights into the mechanisms of protein misfolding and aggregation, which are central to other neurodegenerative diseases like Alzheimer's and Parkinson's. In the prion diseases, misfolded proteins in the form of PrP^{Sc} accumulate and form aggregates that are resistant to normal cellular catabolic processes, with associated (although ill-defined) toxicity leading to neurodegeneration. Similarly, Alzheimer's disease is characterized by the buildup of amyloid-β plaques, and Parkinson's disease involves the accumulation of α-synuclein aggregates. These proteins exhibit prion-like properties, meaning they can propagate misfolding by recruiting and templating normal proteins to adopt their dysfunctional conformation. This prion-like mechanism is central to the progression of these diseases and suggests shared pathogenic pathways across these disorders (119).

Studies have revealed that amyloid-β and α-synuclein can spread in a prion-like manner within the brain, supporting the concept of transmissibility between brain regions (120). Understanding the prion-like behavior of these proteins could not only offer better diagnostic tools but also inform the development of therapeutic strategies aimed at inhibiting the aggregation and propagation of misfolded proteins, which might be effective across various neurodegenerative diseases but also highlight the potential conserved nature of the protein structures.

GENOME-WIDE ASSOCIATION STUDIES

GWAS have become an indispensable tool in prion research, significantly enhancing our understanding of the genetic factors contributing to prion diseases such as CJD, BSE, and atypical prion diseases (121). GWAS allow researchers to analyze genetic variations across the entire genome in large cohorts of affected individuals, facilitating the identification of risk factors linked to disease susceptibility. One of the most notable findings from GWAS in prion research is the association between the *PRNP* gene, which encodes the prion protein, and susceptibility to CJD and other

prion diseases. Studies have shown that specific mutations in *PRNP*, such as the E200K mutation, are strongly correlated with familial forms of CJD. Additionally, another study revealed novel genetic loci associated with prion disease, including variants in genes involved in protein folding and cellular processes, such as *STX6* and *GAL3ST1*, which have been linked to sCJD and other neurodegenerative diseases (122). Although GWAS have not yet been conducted on animal prion diseases like BSE, findings from human prion diseases could be translated to BSE, shedding light on potential genetic factors underlying atypical forms of BSE and the differences between them. By pinpointing these genetic markers, GWAS provide new opportunities for early diagnosis, better understanding of disease mechanisms, and the development of targeted treatments for prion diseases.

THERAPEUTICS

Recent research has provided significant insights into strategies for stabilizing prion proteins to prevent their misfolding and aggregation, which are central to the pathogenesis of prion diseases. A recent study examined how copper ions (Cu^{2+}) affect the behavior of a disease-associated variant of the human prion protein (huPrP23–144), finding that when copper binds to the protein, it changes both how quickly the protein forms amyloid fibrils and the structure of those fibrils. Remarkably, the altered fibrils closely resembled those linked to a genetic form of prion disease caused by the A117V mutation. Copper was shown to bind mainly to specific histidine residues in the protein, which appears to influence how the protein folds. These results highlight the important role that metal ions like copper may play in the development of prion diseases by affecting the misfolding process of prion proteins and future therapeutic intervention (123).

Anti-PrP antibodies have emerged as a promising therapeutic approach for prion diseases, targeting the prion protein (PrP) directly to prevent its misfolding and aggregation. These antibodies can bind to either PrP^{C} or PrP^{Sc}, with the goal of neutralizing or promoting the clearance of the abnormally folded kind and reduction of normal cellular PrP to reduce conversion. A study explored the impact of 145 monoclonal antibodies (mAbs) designed to target both PrP^{C} and PrP^{Sc}. The results showed that mAbs targeting PrP^{C} on the cell surface could effectively eliminate PrP^{Sc}, blocking its conversion into infectious prions and halting their replication. Additionally, these mAbs facilitated the degradation and removal of accumulated prion proteins from infected cells. This research highlights the potential of anti-PrP antibodies as a therapeutic approach, indicating that they could be an effective strategy to slow or halt prion disease progression by neutralizing toxic prion species that are involved in protein misfolding and neurodegenerative damage (124).

A recent clinical trial assessed the safety and potential effectiveness of PRN100, a mAb targeting PrP^{C}, in patients diagnosed with CJD. Six patients were administered intravenous PRN100 at escalating doses, achieving cerebrospinal fluid concentrations of 50 nM. The treatment was well-tolerated, with no significant adverse effects observed. Although all patients showed progressive neurological decline, one patient demonstrated a slower disease progression and distinct patterns of disease-associated PrP, suggesting a possible therapeutic effect of the antibody. Neuropathological analysis of two patients revealed no signs of cytotoxicity. These results provide evidence supporting the potential of anti-PrP^{C} antibodies as a therapeutic option for prion diseases, warranting further investigation into their efficacy (125).

Recent studies have underscored the potential of antisense oligonucleotides (ASOs) as a promising therapeutic strategy for prion diseases. A study showed that ASOs targeting PrP^{C} messenger RNA effectively reduced PrP levels, leading to a decrease in prion protein synthesis. This reduction in protein levels resulted in a significant extension of survival in prion-infected mice, with the treated animals surviving 61% to 98% longer than untreated controls and 55% longer

when administered 120 days postinfection. Moreover, the study demonstrated that ASOs were effective even in the advanced stages of prion-induced neurodegeneration, indicating their potential utility for treating prion diseases at various stages of progression. By targeting the genetic mechanisms driving prion pathogenesis, ASOs offer the possibility of halting or slowing the progression of these fatal neurodegenerative diseases, representing a novel approach in prion therapy (126).

CONCLUSIONS

Despite significant progress in understanding the molecular underpinnings of prion diseases, there are currently no effective treatments or cures for either animal or human prion diseases. Research efforts are focused on exploring therapeutic approaches such as immunotherapies targeting prion aggregation, small-molecule inhibitors that could block prion propagation, and gene therapies aimed at correcting the underlying genetic mutations associated with familial prion diseases. Although these approaches are still in the early stages, they offer hope for future therapeutic intervention.

Further differentiating between the two types of BSE by analyzing their molecular signatures will highlight their potential impact on public health. Surveillance and research continue to investigate the transmissibility, pathogenesis, and risk factors associated with both H- and L-type BSE, with an emphasis on refining diagnostic methods and improving preventive measures to minimize the risks associated with prion diseases in livestock populations. Understanding the distinctions between H- and L-type BSE is crucial for monitoring and controlling BSE outbreaks and ensuring food safety, particularly considering ongoing concerns regarding zoonotic transmission of prion diseases. Nonetheless, prion diseases remain a major challenge due to their high (and inexorable) lethality, the unique and complex nature of prion biology, and the lack of definitive treatments, making them an active area of research in the fields of neurodegeneration and infectious diseases.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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