Prion Zoonoses

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ABSTRACT

Prions are strange, unconventional pathogens composed exclusively of protein. They propagate by templating conversion of a brain protein, PrPC, into an alternative conformation, PrPSc. PrPSc is an amyloid. Prions cause fatal neurodegenerative diseases with exceedingly long incubation times to economically valuable domestic animals: scrapie of sheep and goats, bovine spongiform encephalopathy (BSE, popularly known as “mad cow disease”) of cattle, chronic wasting disease (CWD or “zombie deer disease”) of cervids, or camel prion disease (CPrD). While transmission of prions between different species is restricted by barriers whose molecular underpinnings we are beginning to understand, zoonotic transmission of animal prions to humans has occurred at least once, during the BSE epizootic that ravaged European cattle in the 1980´s. In contrast, no cases of zoonotic transmission have been ever associated to scrapie or CWD. The zoonotic potential of CPrD is still unknown. However, factors such as adaptation of PrPSc prions through intermediate species that cohabit with the primary hosts might result in unexpected breaches of transmission barriers. Implementation of active surveillance programs is an urgent necessity. In this chapter, the main biological and pathological features of animal prion diseases are summarized, together with a brief presentation of the analytical techniques used to diagnose them. A description of the current understanding of the mechanism of prion replication, at the molecular level, is also presented.

Keywords: prions, scrapie, bovine spongiform encephalopathy, chronic wasting disease, camel prion disease.

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1. INTRODUCTION

1.1. PRIONS: STRANGE PATHOGENS MADE ONLY OF PROTEIN

The term *prion* was introduced in 1982 by Stanley Prusiner and defined as a "proteinaceous infectious particle" (Prusiner 1982). Prion is pronounced "/*pri:ɒn/*" (Prusiner SB, many public communications); if pronounced "/*praɪɒn/*", the word refers to a bird, a small petrel of the *Pachyptila* or *Halobaena* genera (Anonymous 2013). Prusiner proposed, and eventually demonstrated, that the pathogen responsible for the infectious neurodegenerative disease scrapie, that affects sheep and goats, is a prion. In 1936, Cuille and Chelle had proven that scrapie was caused by a pathogen with a size typical of a virus (Cuille and Chelle 1938). Given the long period of time, measured in years, between its experimental inoculation and the emergence of clinical signs, it was described as a "slow virus", a taxonomic category created *ad hoc*. However, all efforts to isolate and identify such virus during the following decades were fruitless. Furthermore, evidence accumulated showing that procedures that destroy nucleic acids, such as irradiation with UV light, did not affect scrapie infectivity titers, whereas manipulations that modified proteins, such as treatment with guanidine, did (Prusiner 1998). Prusiner concluded that the agent had to be composed of protein alone, a notion that seemed heretical at the time: how could a pathogen propagate without DNA or RNA? In the following two decades the prion concept gained support and eventually, full acceptance (Prusiner 1998; Aguzzi and De Cecco 2020). Key milestones in this process were the demonstration that knock-out (KO) mice not expressing the prion protein in their brain were completely resistant to prion disease (Prusiner 1998) and the generation of totally recombinant prions (Aguzzi and De Cecco 2020).

Besides ovine and caprine scrapie, prions cause and transmit bovine spongiform encephalopathy (BSE), popularly known as "mad cow disease", chronic wasting disease (CWD) of cervids, and camel prion disease (CPrD). BSE showed the zoonotic potential of prions, as it transmitted in the 1980´s through 2000´s to humans, generating variant Creutzfeldt-Jakob disease (vCJD). About ~300 people died of vCJD and likely many more were silently infected (Requena et al. 2016). Finally, spontaneously generated human prions (*vide infra*) causing a sporadic form of prion disease termed sporadic CJD, have been shown to transmit iatrogenically (Requena et al. 2016) and to cause a localized prion epidemic in Papua New Guinea in the 1950´s termed kuru (Aguzzi and Calella 2009).

2. THE MECHANISM OF PRION PROPAGATION

A mammalian prion is a misfolded conformer of a brain protein termed PrP (prion protein). The normally folded conformer of the prion protein, termed PrP^{C} (cellular isoform of the prion protein) is expressed in many mammalian cells, particularly in the brain. Its function is not fully understood, although it is known to participate in myelination of nervous fibers (Aguzzi and De Cecco 2020). PrP^{C} is a cell membrane protein, tethered to it through a C-terminally attached glucosylphosphatidylinositol (GPI) anchor. It is quite conserved among mammals. Mature PrP^{C} is composed of residues 23-230: the N-terminal 22 residues are cleaved off during maturation. PrP^{C} has two large domains, one globular, in turn made up of three α helices and a short β sheet, and another one highly flexible if not disordered (Aguzzi and Calella 2009). Each of these two domains comprises approximately one half of PrP^{C}. The prion form of PrP, termed PrP^{Sc} (scrapie isoform of the prion protein) has a completely different fold: its C-terminal domain has refolded to a completely flat succession of short β strands connected by short loops (Caughey et al. 2022). These flat domains stack to form a "parallel in-register beta stack" (PIRIBS), forming long amyloid fibrils (Fig. 1). Both PrP^{Sc} and PrP^{C} are variably glycosylated, featuring two, one or no glycans attached to the protein (Prusiner 1998).

Prion propagation consists of a PrP^{Sc}-templated conversion of PrP^{C} into PrP^{Sc}. From the perspective of PrP^{C}, the outermost surface of a PrP^{Sc} is a template. The PrP^{Sc} stack features hydrogen bonds between -C=O and HN- groups in residues located in stacked β strands. But in the outermost surface there is a deficit of such hydrogen bonds, rendering it a "velcro-like" template ready to trap and mold any isosequential PrP stretch coming close to it. And that is precisely what the ~95-124 unfolded stretch of PrP^{C} is (Fig. 1), so templating and conversion of that stretch occurs easily. Once this templating event has concluded, the remaining ~125-230 globular domain of PrP^{C} has to unfold and then refold onto the PrP^{Sc} templating surface.

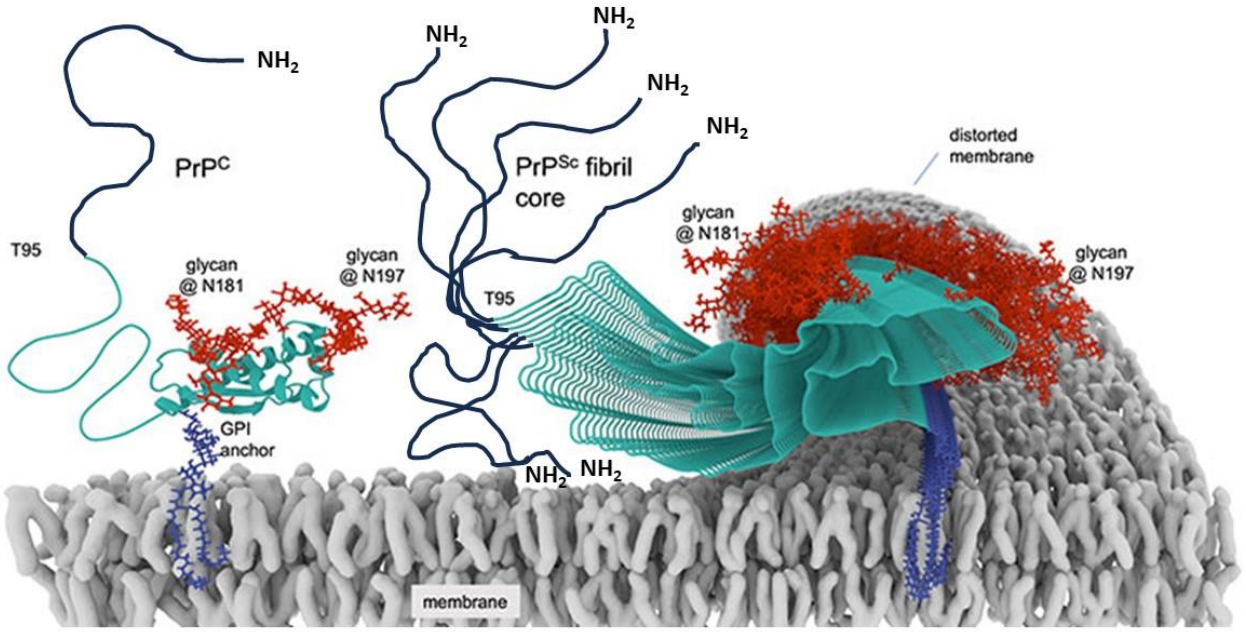

Fig. 1: Propagation of prions: conversion of PrP^{C} to PrP^{Sc}. PrP^{C} (left) encounters a PrP^{Sc} assembly (right) on a neuronal membrane. The amyloid core of PrP^{Sc} (cyan) consists of stacked ~95-230 sections bound together by hydrogen bonds. Each flat, extended PrP^{Sc} monomer is "sandwiched" between two identical PrP^{Sc} monomers, in which each amino acid residue lies on top of and under an identical residue. The extreme N-terminal stretch of PrP^{C} (23-124) which contains many Pro and Gly residues, incompatible with a β-sheet, does not change its conformation and remains highly flexible (in black). GPI anchors are depicted in blue and glycans in red. Modified with permission from Kraus et al. (2021).

Details of how this takes place are still not known. An atomistic model of the entire conversion process was proposed, but it was based on an inaccurate structural model of PrP^{Sc} (Spagnolli et al. 2019). However, its main features are likely to be correct. Once a PrP^{C} unit has been transformed into PrP^{Sc}, the templating cycle can continue *ad infinitum* as long as there is a supply of PrP^{C}. This is why PrP KO mice are refractory to prion infection (Prusiner 1998).

PrP^{Sc} is very resistant to proteases; to be more precise, its compact ~95-230 amyloid core is. Thus, when experimentally treated with proteinase K (PK), the flexible N-terminal ~23-94 tails "dangling" from the amyloid core stack (Fig. 1) are destroyed, but the core itself resists the treatment, remaining as a truncated form of PrP^{Sc} termed PrP27-30 (Aguzzi and Calella 2009) (Fig. 2).

Such unusual resistance to PK is used as the basis to detect prions in animal samples. Resistance to proteases is also key for propagation of PrP^{Sc} prions between animals. When an infected animal dies, PrP^{Sc} prions in its carcass resist autolysis, and being resilient to high temperatures and desiccation, they remain

in the soil and grass, from where they can be ingested by other members of the herd. Ingested prions partially resist enzymes in the digestive tract (Aguzzi and Calella 2009).

From the gut, prions traverse into the subendothelial space, particularly in Peyer patches, via transcytosis across M cells (Fig. 3). Some are transferred to cells of the secondary lymphatic organs (SLO). Conventional dendritic cells play a key role in transfer of prions to follicular dendritic cells. These cells express PrP and provide a first site for PrP^{Sc} replication. Eventually, some can transfer to nerve endings. Then, by retrograde transport, they can move to the brain (Fig. 3). All this takes a substantial amount of time, and propagation in and across the brain, an additional portion, until damage to the brain becomes apparent through clinical signs. During this last phase, propagation is exponential. When the infected animal dies, the cycle begins again.

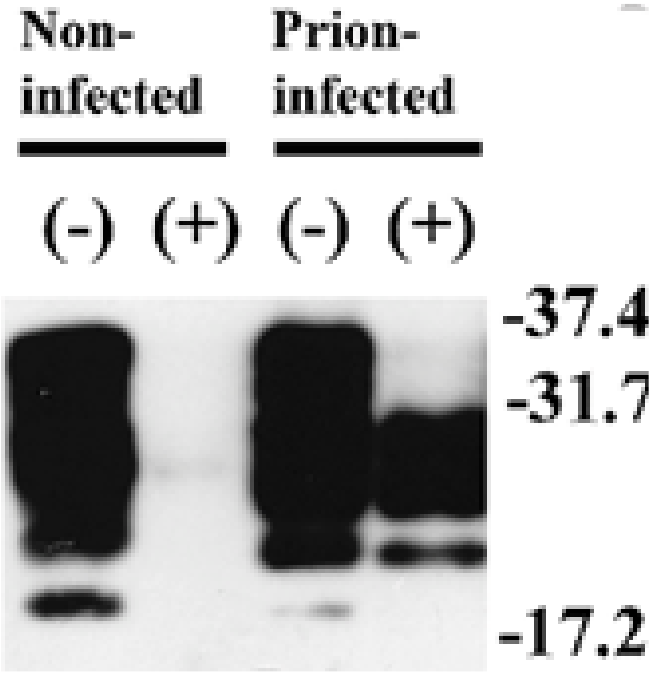

Fig. 2: Western blot of brain homogenate samples from animals not infected or infected with a prion disease. Samples were (+) or not (-) treated with PK and probed with a PrP-specific antibody. Approximate molecular masses are shown. The appearance of different bands is the consequence of di-mono- and nonglycosylated populations of PrP. PrP^{C} exhibits some degree of spontaneous fragmentation in the absence of PK. Adapted, with permission, from Sakudo and Onodera (2011).

Of note, in some prion diseases involvement of cells of the SLO is particularly important, and peripheral, extra-encephalic prions can reach into milk, urine and feces, which become additional sources of infectivity (Mabbott 2017).

3. PRION STRAINS AND TRANSMISSION BARRIERS

While a given PrP sequence results in a single PrP^{C} conformation, dictated by Anfinsen´s principle, it can result in not one but several PrP^{Sc} conformations, all sharing the same basic architecture but exhibiting minor structural nuances (Hoyt et al. 2022). Such PrP^{Sc} variants are known as strains. They maintain their unique structural characteristics as they propagate and give rise to distinct biological properties and pathological phenotypes (*vide infra*). Why different PrP^{Sc} strains cause diseases with distinct phenotypes is not fully understood, but it is a fact that different strains accumulate in different brain areas. Transmission of prions between species involves mismatches between a sequence of PrP^{Sc} and that of the host PrP^{C}. This often leads to steric hindrances, v.g., if the mismatch involves a larger or charged residue in the host´s PrP^{C} that will just not fit into the PrP^{Sc} template (Kraus et al. 2021). This creates a transmission barrier. Since different PrP^{Sc} strains of a given species exhibit conformational differences, transmission barriers with other species can be mitigated or accentuated for different strains (Aguzzi and Calella 2009).

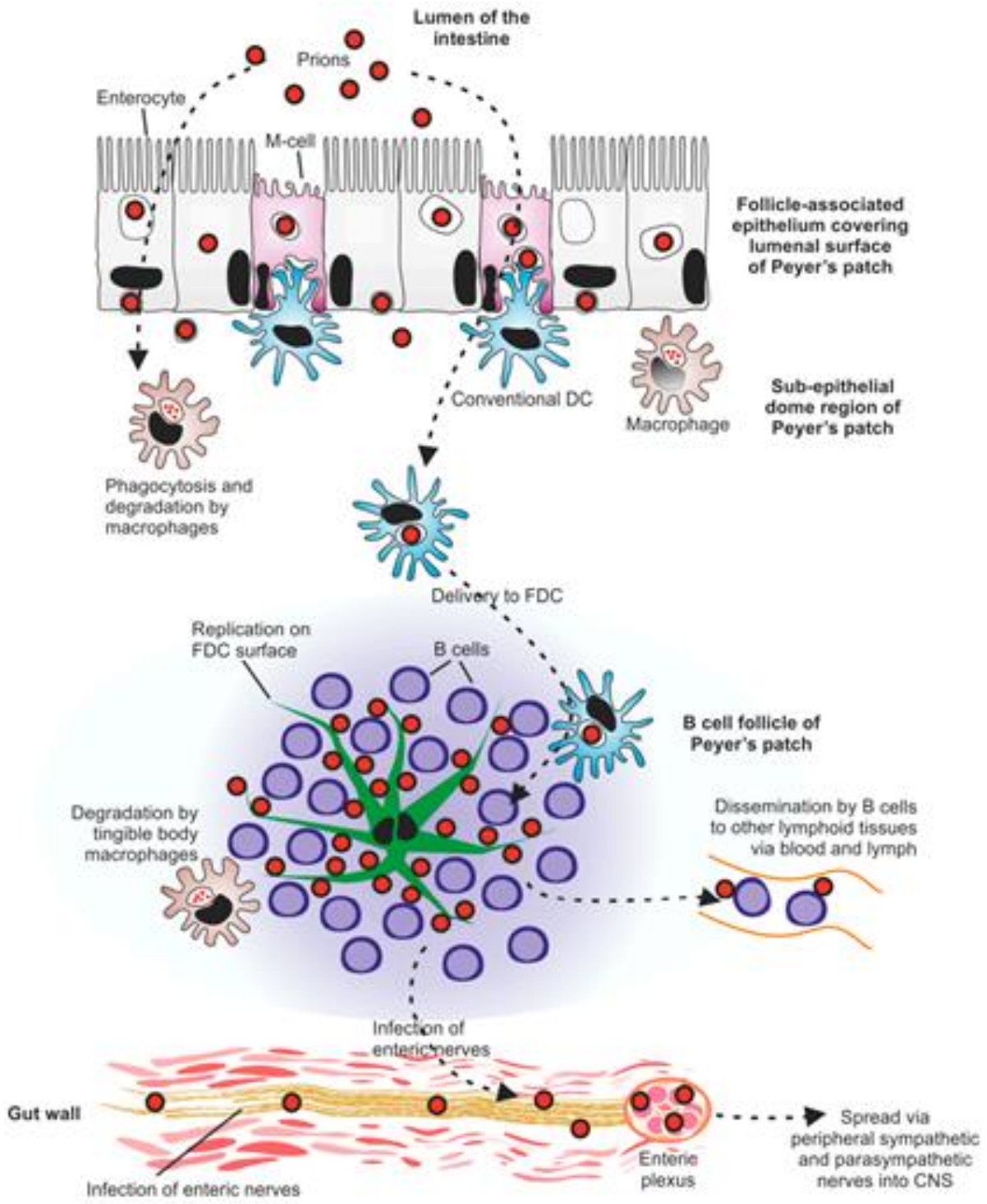

Fig. 3: Passage of prions from the gut to the brain. Prions traverse the intestine mainly by transcytosis through M cells located in Peyer patches. They are subsequently phagocytosed by conventional dendritic cells, that deliver them to other cells of the SLO, including follicular dendritic cells, where prions propagate and accumulate. Eventually, prions reach enteric nerves and are transported to the central nervous system. Reproduced with permission from Mabbott (2017).

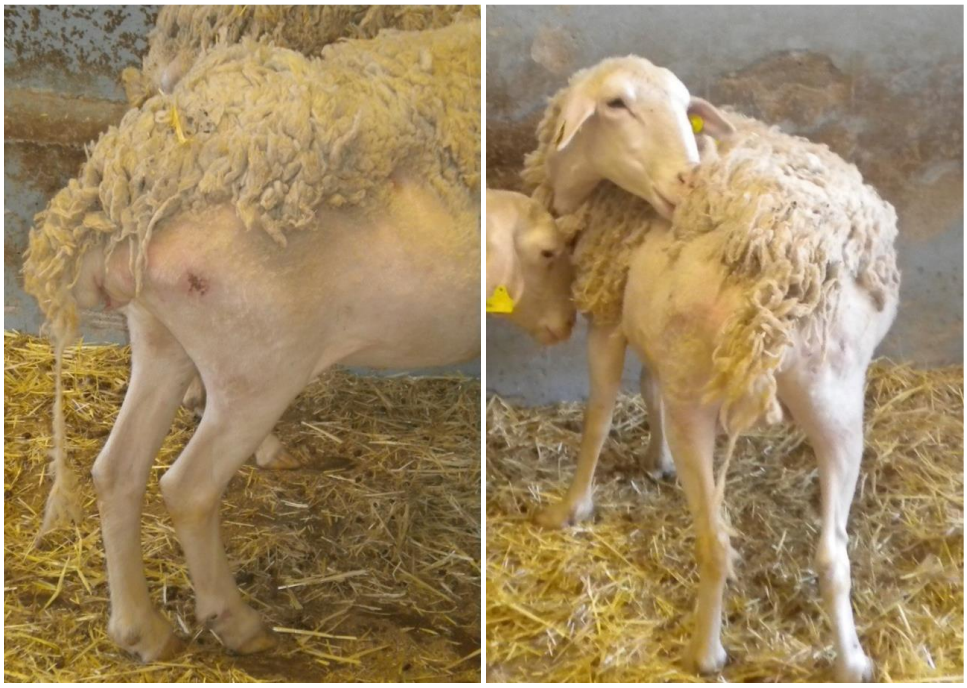

Fig. 4: Clinical manifestations of scrapie. Left: alopecia and cutaneous lesion. Right: scratching. Images by Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, University of Zaragoza, Spain.

4. SCRAPIE

Ovine and caprine scrapie was first documented in England in 1732. Since then, it has spread to become the most widely distributed prion disease worldwide. Nowadays, it is considered as the prototype and model for the study of other prion diseases (Aguzzi and Calella 2009).

Transmission of the classical form of scrapie occurs mainly horizontally, through environmental contamination (Andreoletti et al. 2002) and possibly vertically, via intrauterine transmission (Foster et al. 2013). Animals affected naturally by classical scrapie are usually between 2 and 5 years, with a life expectancy after the onset of the first clinical signs of 1 to 6 months (Collinge and Clarke 2007). Most frequent clinical signs are changes in behavior, such as separation from the herd, loss of body condition, exaggerated response to external stimuli, bruxism with constant lip movements, changes in locomotion patterns (ataxia), head tremors, and the appearance of intense pruritus leading to wool loss (Fig. 4) (Clark and Moar 1992).

In 1998 an atypical form of scrapie, named Nor98, was observed in Norway (Benestad et al. 2008). It differs clinically and epidemiologically from classical scrapie and has its own biochemical and histopathological characteristics. It is also distributed worldwide and has a similar incidence to classical scrapie. Animals suffering from atypical scrapie are usually individuals over 5 years of age and it is common to see isolated cases in the herd (Fediaevsky et al. 2008). Due to its epidemiology, it is considered a non-infectious form of the disease in which a characteristic strain of PrP^{Sc} appears spontaneously in the brain, an extremely rare occurrence of spontaneous misfolding of PrP^{C} to PrP^{Sc} (Benestad et al. 2008; Vidal et al. 2022). Atypical scrapie has been confirmed in areas considered free of classical scrapie, such as Australia (Cook et al. 2016). Common atypical scrapie clinical signs include progressive ataxia, tremors, loss of body condition, circular movements and visual impairment (Simmons et al. 2009). No pruritus and therefore no alopecia have been documented (Acin et al. 2021; OIE, FAO 2022).

Numerous polymorphisms of the PRNP gene (which encodes the PrP^C protein) have been described in different species and have a major impact on the development of naturally occurring prion diseases (Hunter 1997), likely influencing the conversion of PrP^C to PrP^{Sc} (Bossers et al. 1997). Polymorphisms in codons 136, 154 and 171 play an important role in the susceptibility to scrapie. Codon 136 can encode the amino acids valine (V), alanine (A) or threonine (T); codon 154 encodes arginine (R), histidine (H) or leucine (L) and codon 171 can code for arginine, histidine, glutamine (Q) or lysine (K). However, out of all possible alleles, only five of them appear with a high frequency: A136R154Q171 (original gene variant, abbreviated as 'ARQ'), ARR, ARH, AHQ and VRQ (Hunter 1997; Goldmann 2008).

Ewes expressing the VRQ or ARQ alleles have a high vulnerability to classical scrapie, whereas the expression of the ARR allele confers resistance. In addition, the ARR haplotype has a dominant effect, and both homozygous and heterozygous animals are at lower risk for this prion disease (Belt et al. 1995). This genetic knowledge has been used for years to genetically select sheep flocks to achieve greater natural resistance to classical scrapie and thus reduce its incidence. But it should be noted that no fully resistant genotype has been detected. Additionally, it has been observed that atypical scrapie appears more frequently in sheep with genotypes associated with a higher resistance to classical scrapie along with homozygosity for phenylalanine at codon 141, while individuals carrying the VRQ allele rarely develop the disease (Tranulis et al. 2011).

Several polymorphisms associated with scrapie susceptibility have also been reported for the goat PRNP gene. Specifically, polymorphisms H143R, R154H, R211Q and Q222K represent an increase in the resistance to classical scrapie, although R154H has been associated with increased susceptibility to atypical scrapie (Holko et al. 2005; Lacroux et al. 2014). Detailed PRNP sequence studies of Pakistani goats have been carried out, given the economic importance of these animals as sources of milk and meat in this country (Hassan et al. 2016).

5. BOVINE SPONGIFORM ENCEPHALOPATHY

BSE was first diagnosed in the United Kingdom in 1986 (Wells et al. 1987). Shortly after, it spread and caused one of the most significant food crises in Europe in recent decades. Animals infected with BSE have incubation periods of 4 to 5 years and exhibit clinical signs similar to those observed in sheep with scrapie, including emaciation, alopecia, apprehension, lethargic or aggressive behavior, hypersensitivity to stimuli, and abnormal movements (Kobold et al. 2006).

Several hypotheses have been formulated regarding the origin of BSE, but the most widely accepted one has been the practice of feeding cattle with meat and bone meal contaminated with infectious prions (Wilesmith et al. 1991). This led to the implementation of a series of measures by different countries to break the transmission cycle of this disease. Thanks to these efforts, its incidence was drastically reduced, although it has not been completely eradicated.

The presence of PrP^{Sc} in animals infected with BSE, unlike scrapie, is mainly limited to the nervous system. However, low infectivity has been described in the small intestine (Peyer's patches), distal ileum, jejunum (Hoffmann et al. 2011), and tonsils (Wells et al. 2005). Moreover, infectivity has been detected in skeletal muscles due to the centrifugal spread of the agent through nerves via motor and/or sensory pathways to muscle tissues. It was important to define specific risk materials to prevent the entry of BSE-contaminated materials into the food chain (Okada et al. 2014).

BSE has demonstrated a great capacity for transmission to other species (Bruce et al. 1994). During the 1980s, it spread to humans, leading to the emergence of vCJD (Bruce et al. 1997). It was also detected in cats and zoo animals, resulting in feline spongiform encephalopathy (FSE), and exotic ungulate encephalopathy (EUE) (Sigurdson and Miller 2003). In 2005, the first case of natural BSE in goats was detected in France (Eloit et al. 2005), repeated a year later in the United Kingdom (Jeffrey et al. 2006).

These studies suggested that goat BSE could pose a potential risk to human health, necessitating improvements in control strategies.

In 2004, two new neuropathological and molecular phenotypes of BSE were detected, classified into two groups based on their biochemical and biological characteristics. The L-type BSE or L-BSE was detected for the first time in Italy (Casalone et al. 2004). Affected animals showed significant differences in the distribution of the encephalic lesions compared to animals infected with classical BSE (C-BSE). On the other hand, H-type BSE (H-BSE) was described for the first time in France (Biacabe et al. 2004). Currently, atypical BSE cases are still reported in several European countries (OIE, FAO. 2022). These cases are diagnosed in adult cattle and their origin is unknown, although it has been proposed that they could be sporadic, as proposed for atypical scrapie. Polymorphisms of the PRNP gene described in cattle (W84R, G100S, K113R, V115M, H143R, S146N, and N177S) have little impact on susceptibility or resistance to BSE (Seuberlich et al. 2010).

6. CHRONIC WASTING DISEASE

CWD affects different members of the Cervidae family, especially elk, moose, and various species of deer. CWD was first identified in captive mule deer (*Odocoileus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) in the late 1960s in Colorado, United States (Miller et al. 2000). Soon after, the disease was identified in contiguous Wyoming, Nebraska, and South Dakota, affecting captive and free-ranging populations (Williams and Miller 2002). Surveillance programs suggested that CWD was endemic among free-ranging deer and elk in this region of North America, indicating that CWD had been spreading through wild cervid populations within this endemic area for decades before its detection. The high prevalence of CWD in some states of the U.S. is a major cause for concern (DeVivo et al. 2017). CWD cases have been reported in twenty-six U.S. states, three Canadian provinces (Rivera et al. 2019), three Scandinavian countries (Tranulis et al. 2021) and two South Korean provinces (Lee et al. 2013). Natural migrations of free-ranging populations and commercial exports contributed to a fast geographical expansion. Epidemiological investigations revealed that CWD cases in South Korea were imported from Canadian farms. In 2016, CWD was also identified in wild reindeer and moose in Norway, followed by the detection of more cases in a semi-isolated reindeer population (Benestad et al. 2016). In an attempt to control the spread of cases in that area, the Norwegian authorities took the drastic decision to cull this entire reindeer population. Testing resulted in 18 positive cases out of 2400 post-culling samples (Tranulis et al. 2021). Intensive surveillance enabled detection of isolated cases in Sweden and Finland. Although the origin of CWD cases in Europe remains unclear, it does not seem to be related to the outbreaks in North America (Miller et al. 2000). No cases of CWD have been described in Pakistan.

Histopathological features vary among the cervid species and the geographical distribution of the populations. Overall, CWD-infected animals present extensive deposition of PrP^{Sc} in lymphoid tissues, which are detectable in the early stages of the disease, and in the central nervous system (Sigurdson et al. 1999). The incubation period and disease progression are also highly variable and are associated with the species, the route of infection, the dose of infectious agents, and the genetic background (Otero et al. 2021). During progression of the disease, animals usually show loss of body weight, hypersalivation, and behavioral changes such as dropped head and ears, and loss of fear of humans. At advanced clinical stages, animals present incoordination and a decline of the body condition (Moreno and Telling 2018).

CWD transmission is highly efficient, and horizontal transmission has been proposed as the main mechanism of infection. CWD prions have been found in saliva (Henderson et al. 2013), urine (John et al. 2013), blood (Mathiason et al. 2006), feces (Pulford et al. 2012), and lymphoid tissues (Benestad et al. 2016). CWD PrP^{Sc} prions persist in the environment for years. They contaminate the soil (Kuznetsova et al.

2014), grazing areas, and water sources (Nichols et al. 2009). Additionally, CWD prions have also been experimentally transmitted from doe to fawn, indicating that vertical transmission is also a possible route of infection (Nalls et al. 2013).

To date, no natural transmission of CWD to humans has been described. Nonetheless, experimental studies have successfully transmitted CWD to various animal species that cohabitate with cervids, such as cattle, sheep, goats, ferrets, minks, raccoons, and mice, indicating that CWD entails a potential risk of cross-species transmission and rising concern about its zoonotic potential (Kurt and Sigurdson 2016).

7. CAMEL PRION DISEASE

In 2018, a prion disease affecting dromedaries (*Camelus dromedarius*) was detected in Algeria. It is estimated that 3.1% of the dromedaries slaughtered in Ouargla between 2015 and 2016 had presented clinical signs compatible with prion disease including weight loss, behavioral abnormalities, tremors, hyperexcitability, abnormal movements of the neck and head, ataxia, falls, and difficulty getting up (Babelhadj et al. 2018). Diagnosis was confirmed following the observation of spongiform degeneration and PrP^{Sc} deposition in the central nervous system of affected animals. It has been demonstrated that the PrP^{Sc} prions causing this disease have biochemical characteristics that are different from those of BSE and scrapie (Babelhadj et al. 2018). The presence of PrP^{Sc} in lymphoid tissues of affected animals suggests the contagious nature of CPrD, although the origin of the disease is still unknown. It was suggested that CPrD could have originated from sheep scrapie since dromedaries are often raised alongside sheep and share common pastures. However, scrapie has not been reported in Algeria. The nomadic herding of dromedaries could have contributed to the spread of the disease at long distances (Babelhadj et al. 2018).

Pakistan has around 1.1 million camel heads, being one of the ten biggest camel producer countries in the world. These animals are an important source of milk, meat and transportation (Faraz et al. 2019). In Pakistan, camel production systems primarily rely on sedentary regimes, where dromedaries are raised from birth to finishing (Faraz et al. 2021). Camels are a vital species for millions of people worldwide. For this reason, attention and investigation are required when a prion disease emerges in a new species and new geographical areas. Implementing a surveillance system and improving the diagnostic capacity for prion diseases in countries where dromedaries are an important part of the domestic livestock would control CPrD and minimize zoonotic risks.

8. ZOONOTIC POTENTIAL OF PRIONS

For decades, it was known that scrapie affected sheep; however, the zoonotic potential of animal prion diseases was considered negligible. This perception dramatically changed in the 1990s, when the emergence of vCJD was associated with the outbreak of BSE in cattle. This event triggered a public health crisis in Europe and demonstrated the zoonotic potential of animal prion diseases (Will et al. 1996; Bruce et al. 1997).

As mentioned, the transmission of prions between different species is governed by a transmission barrier. This refers to a natural resistance to propagate prions from other species and arises mainly from differences in the primary structure of prion protein (Béringue et al. 2008a). It should be noted that the transmission barrier is not absolute, and under certain circumstances, prions can adapt and overcome it. This likely involves a slight conformational change in the templated product to avoid any hindrance(s) posed by PrP sequence differences.

As a result of the transmission of C-BSE to humans and the emergence of vCJD, the transmission barrier between different animal prion diseases and humans has been extensively studied to assess their zoonotic

potential (Torres et al. 2016). The use of transgenic mice expressing the human PrP enabled to relate the outbreak of C-BSE with vCJD and to study the zoonotic risk of the atypical variants of BSE. These studies showed that L-BSE presented equal or greater virulence than C-BSE, suggesting that this prion disease entails an important zoonotic risk. On the contrary, H-BSE presented a high transmission barrier, indicating that this variant poses a lower zoonotic risk (Béringue et al. 2008b).

Regarding scrapie, whereas epidemiologic studies have not associated exposure to small ruminant products as a risk factor for developing CJD, experimental transmission of classical scrapie isolates to non-human primates has raised concern about the zoonotic potential (Comoy et al. 2015). On the other hand, transmission of classic and atypical scrapie isolates to transgenic mice expressing human PrP show non-conclusive results. Successful transmission of scrapie isolates to humanized mice depends on multiple factors such as polymorphisms in the human PrP sequence. Overall, results showed subclinical infections or inefficient transmission on the first passage but clear infectivity after serial passages, suggesting some zoonotic potential of scrapie (Torres et al. 2016).

Infectivity of several CWD isolates has been tested in humanized mice expressing different human PrP polymorphic variants, and all of them failed to show clinical symptoms or accumulation of CWD prions in the brain (Kong et al. 2005; Wilson et al. 2012; Kurt et al. 2015). Transmission of CWD has also been assessed in non-human primates with contradictory results. While one experiment demonstrated transmission of CWD to cynomolgus macaques, another failed to show infectivity by intracerebral inoculation in animals of the same species (Race et al. 2014; Moreno and Telling 2018). Altogether, these data have prompted concern about the risk of CWD for public health.

9. METHODS TO DETECT PRIONS

The diagnosis of prion diseases usually involves a combination of clinical and laboratory diagnostic methods. Clinical diagnosis is not definitive, as the clinical signs are nonspecific and similar to those of other pathologies. Thus, definitive diagnosis is always postmortem (Wilesmith et al. 1992; Konold et al. 2004; Williams 2005).

Traditionally, the diagnosis of prion diseases has been based on histopathological analysis of central nervous system tissue samples by light microscopy in search of characteristic histological lesions such as vacuolization, spongiform change (Fig. 5), gliosis, neuronal degeneration and loss, and amyloidosis (Wells and McGill 1992; Ligios et al. 2002). For example, BSE is characterized by the presence of vacuolization mainly in the medulla oblongata at the level of the obex (Jeffrey and González 2004) and also in the central gray matter, rostral colliculus, and hypothalamus (Simmons et al. 1996; Ganley et al. 2015). Both classical and atypical scrapie show similar lesions, although they differ in the distribution pattern of vacuolization. Thus, while classical scrapie is usually characterized by bilateral and symmetrical vacuolization in the spinal cord, brainstem, and hypothalamus, in atypical scrapie there is no vacuolization in the brainstem, being more frequent in the cerebellar and cerebral cortices, and in the basal ganglia (Wood et al. 1997).

However, lesions are not always observed. Therefore, the most commonly used laboratory diagnostic methods are currently based on the detection of PrP^{Sc} accumulation in tissue samples, as it occurs prior to the appearance of lesions (Grassi et al. 2008). Western blot (Fig. 2) and immunohistochemistry (IHC) (Fig. 5) are two gold standard diagnostic techniques based on the detection of proteinase K-resistant fragments of PrP^{Sc} by means of specific antibodies. Western blot allows the detection of PrP^{Sc} and the characterization of prion strains by the different electrophoretic patterns as a result of their different degrees of glycosylation and sites of proteolytic cleavage (Grassi et al. 2008; Orge et al. 2021). On the other hand, IHC allows the detection of PrP^{Sc} deposits *in situ* and identification of their cellular location, tissue distribution, and morphological characteristics (Grassi et al. 2008; Orge et al. 2021). In classical scrapie,

PrP^{Sc} accumulations are mainly observed in the medulla oblongata, at the level of the obex, both intraneuronal and outside the neurons (González et al. 2003), as well as in lymphoid tissues associated with the third eyelid, palatine tonsils or rectal mucosa (Espenes et al. 2006). The PrP^{Sc} deposits in lymphoid tissue in classical scrapie can be used for the detection of preclinical non-symptomatic infected sheep (Monleón et al. 2011). However, sheep with classical scrapie-resistant genotypes hardly accumulate PrP^{Sc} in lymphoid tissues (Jeffrey et al. 2002; Ersdal et al. 2003). In atypical scrapie, on the other hand, PrP^{Sc} deposition is mainly localized in the cerebral cortex and cerebellum, at the perineuronal level, and in the neuropil (Benestad et al. 2008), without occurrence in peripheral lymphoid tissue (Moore et al. 2008).

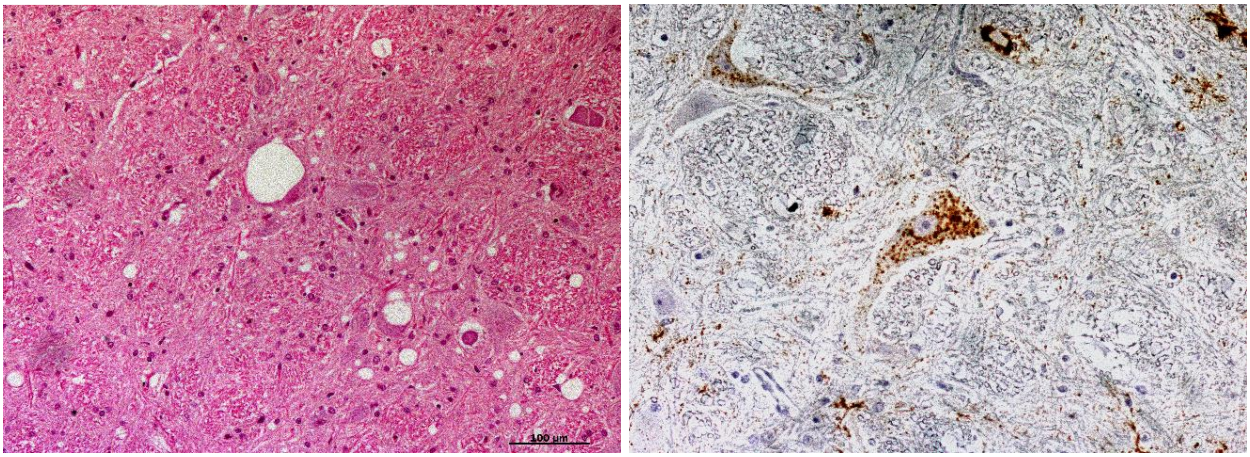

Fig. 5: Brain histopathology of prion diseases. Left: spongiform change. Right: PrP^{Sc} deposits stained with a PrP specific antibody (brown signal). Intra- and extracellular deposits are seen. Images by Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, University of Zaragoza, Spain.

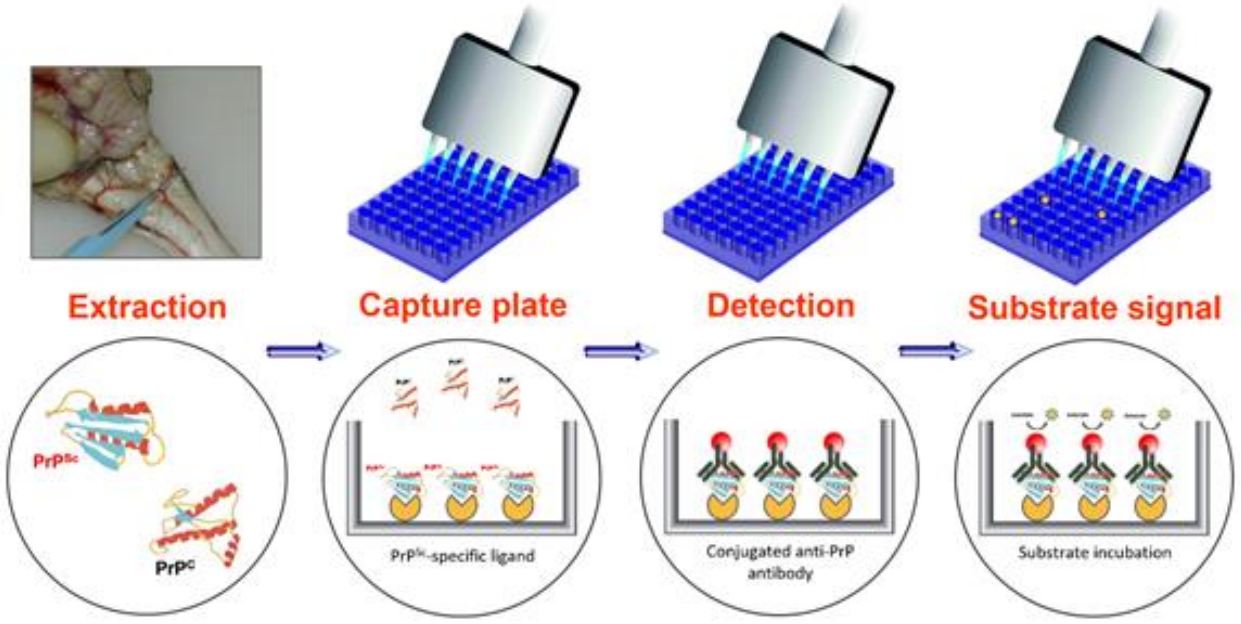

Fig. 6: Scheme of a rapid (ELISA) test to detect prion disease. After sampling, tissue is homogenized and analyzed with a commercial kit. The structure of PrP^{Sc} shown is not realistic.

There also exist rapid tests, which stand out for their usefulness in surveillance and eradication programs since they allow the diagnosis of a large number of animals in a short period due to their speed and simplicity (Fig. 6). In general, these tests are based on immunodetection of PrP^{Sc}, for which most of them include a first step of distinction between PrP^{C} and PrP^{Sc} based on their different biochemical properties, especially the relative resistance of PrP^{Sc} to digestion by PK. In addition, all rapid tests include a denaturation step of PrP^{Sc} for subsequent detection by anti-PrP antibodies. A positive result obtained with these tests is not definitive, and it is necessary to confirm it by Western blot or IHC (Esteves et al. 2021).

10. CONCLUDING REMARKS

The BSE epizootic, with transmission of prions to humans (vCJD), was a hard awakening. While epidemiological and experimental studies show that the zoonotic potentials of scrapie, CWD and CPrD are limited, factors such as adaptation of PrP^{Sc} prions through intermediate species that cohabit with the primary hosts might result in unexpected breaches of transmission barriers. Implementation of active surveillance programs is an urgent necessity.

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