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Animal prion diseases: A review of intraspecies transmission

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Abstract

Animal prion diseases are a group of neurodegenerative, transmissible, and fatal disorders that affect several animal species. The causative agent, prion, is a misfolded isoform of normal cellular prion protein, which is found in cells with higher concentration in the central nervous system. This review explored the sources of infection and different natural transmission routes of animal prion diseases in susceptible populations. Chronic wasting disease in cervids and scrapie in small ruminants are prion diseases capable of maintaining themselves in susceptible populations through horizontal and vertical transmission. The other prion animal diseases can only be transmitted through food contaminated with prions. Bovine spongiform encephalopathy (BSE) is the only animal prion disease considered zoonotic. However, due to its inability to transmit within a population, it could be controlled. The emergence of atypical cases of scrapie and BSE, even the recent report of prion disease in camels, demonstrates the importance of understanding the transmission routes of prion diseases to take measures to control them and to assess the risks to human and animal health.

Keywords: Animal prion diseases, Scrapie, Bovine spongiform encephalopathy, Chronic wasting disease, Transmissible spongiform encephalopathies.

Introduction

Prion diseases are a group of neurodegenerative disorders that affect humans and several animal species. These diseases are also known as transmissible spongiform encephalopathies (TSE) due to the microscopic changes they produce in the central nervous system (CNS) and their capability for transmission among susceptible individuals. Diseases within this group include Creutzfeldt Jakob disease (CJD) in humans, scrapie in sheep and goats, and bovine spongiform encephalopathy (BSE) in cattle, among others. All of them have a chronic course with neurological manifestations and culminate in the death of affected individuals since there is no curative treatment for these pathologies.

The relevance of animal prion diseases has been changing over the years. BSE caused strong changes in animal production at the end of the 20th century, but its significance decreased with the implementation of measures to control it. However, BSE and other prion diseases are still being diagnosed worldwide, and a novel disorder designated as camel prion disease (CPD) was recently reported in dromedary camels from Algeria (Babelhadj *et al.*, 2018). These findings indicated that prion diseases remain relevant for animal production and public health.

Although the knowledge of animal prion diseases has increased drastically in the last years, many aspects remain poorly understood despite being studied in depth. Information about the spread of animal prion diseases became necessary for their control. This work aimed to compile and analyze information about the transmission routes of animal prion diseases within susceptible populations.

Animal prion diseases

Animal prion diseases have been known since 1732, when scrapie was described in a merino sheep in Spain, and later it was well documented in sheep from Great Britain (Liberski, 2012). During the 1960s, scrapie-like diseases were described in mink and deer in North America, later known as transmissible mink encephalopathy (TME) and chronic wasting disease (CWD) respectively. But it was in 1986 when animal prion diseases became more relevant. In that year, it was the first report about a disease similar to scrapie affecting cattle. That new disease was called BSE (Wells *et al.*, 1987), and its impact was more significant than other prion diseases due to the economic importance of cattle. Interest in these pathologies increased significantly in 1996 when BSE was linked to a variant of the CJD in humans and was therefore considered a zoonosis (Will *et al.*, 1996; Bruce *et al.*, 1997). Because of

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the risk to animals and humans, strict measures to reduce the transmission of BSE were successfully implemented, and the number of new cases has decreased dramatically in recent years.

Although prion diseases have been known for a long time, the etiological agent was characterized at the end of the 20th century when the prion hypothesis was postulated (Prusiner, 1982). It was proposed that scrapie is caused by a misfolded isoform of normal cellular prion protein (PrP^C), which is found constitutively in nucleated cells of all superior species with a higher concentration in the CNS. The misfolded form was initially called protease resistant prion protein (PrP^{res}) or scrapie prion protein (PrP^{Sc}). The conversion of PrP^C to PrP^{res} is a post-translational process that has not been completely elucidated yet.

PrP^{res} can be detected by several procedures. Immunoblotting, enzyme-linked immunosorbent assay, and immunohistochemistry are the most widely used methods in research and diagnosis (Gavier-Widén *et al.*, 2005). New techniques have been developed, such as protein misfolding cyclic amplification (PMCA) and real time-quaking induced conversion (RT-QuIC). These consist of reproducing the conversion of PrP^C to PrP^{res} *in vitro*, using the PrP^{res} present in infected samples. PMCA and RT-QuIC allowed them to advance in the knowledge of prion diseases since they have a higher sensitivity than other methods (Eraña *et al.*, 2020).

Considering all animal prion diseases, scrapie is the best known. It has been the prototype for studying prion diseases, and it is the most widespread, with the highest number of new cases reported. Additionally, although there has been no evidence of scrapie transmission to humans, it has been postulated as a potential risk for humans since it has been transmitted to primates and transgenic mice overexpressing human native PrP^C (Cassard *et al.*, 2014; Comoy *et al.*, 2015). Beyond its hypothetical risk to other species, scrapie spread among small ruminants creates commercial barriers for sheep and goats, and their derived products.

Nowadays, CWD has become the most worrisome animal prion disease because it has been shown to be maintained in wildlife animals and has spread across large areas. Initially confined to some states in the United States of America (USA) and Canada, CWD cases have been recently detected in other regions of both countries (Rivera *et al.*, 2019) and more recently in Norway, Sweden, and Finland (Benestad *et al.*, 2016; Koutsoumanis *et al.*, 2019). Furthermore, it has been proposed that CWD could represent a zoonotic disease and, therefore, a potential risk for public health (Hannaoui *et al.*, 2017).

Prion protein

The primary structure of PrP^C consists of 256 amino acids in sheep, which varies slightly among animal species. The secondary structure of PrP^C is mainly helical (40% α -helix and 3% β -sheet) when it is solubilized in detergents in the absence of cations.

The formation of the PrP^{res} leads to a modification of secondary structures (30% α -helix and 45% β -sheet), which modify its biochemical characteristics (Pan *et al.*, 1993). While PrP^C is completely degraded by proteases, PrP^{res} is partially resistant. For this reason, PrP^{res} accumulates in neurons and glial cells, causing vacuolization of gray matter with a microscopic “spongiform” change that characterizes prion diseases. PrP^C is encoded in the *PRNP* gene, and it is expressed in high levels in the CNS, mainly associated with neurons and astrocytes, with lower levels present in oligodendrocytes and microglia. It can also be found in other tissues such as muscle, peripheral nervous system (PNS), or lymphoid tissue, but in less quantity than in the CNS (Watts *et al.*, 2018). Although the function of PrP^C is unknown, it has been shown that the absence of PrP^C in knockout mice makes the animal resistant to prion diseases (Weissmann and Flechsig, 2003). Therefore, the expression of this protein in the cells of susceptible hosts is necessary for the development of prion diseases. Unlike other prion diseases, an association between the risk of scrapie infection and some sheep genotypes has been described. This genetic susceptibility to the disease is given by the polymorphism of *PRNP* gene and mainly to variations in the amino acids encoded in three codons: 136, 154, and 171. From these combinations arise 5 alleles (VRQ, ARQ, AHQ, ARH, and ARR) with 15 possible genotypes (Goldmann, 2008). Among these, the VRQ/VRQ genotype confers high susceptibility to scrapie, while ARR/ARR is associated with low susceptibility (Baylis *et al.*, 2004). In this way, scrapie infection depends on exposure to the infectious agent and the host's genetic susceptibility. These findings led to the development of breeding programs in Europe intending to eliminate those individuals with genotypes susceptible to scrapie (Melchior *et al.*, 2010).

In goats, around 50 polymorphisms of the *PRNP* gene have been described. However, it has not been determined with the same precision as in sheep if there is a direct correlation between goat genotypes and the susceptibility of contracting scrapie (Greenlee, 2019). For this reason, no breeding programs have been developed in goats, as in the case of sheep.

In other TSE, genetic predisposition has not been demonstrated. Some studies in cattle revealed that regions outside the coding region of the *PRNP* gene are associated with variations in disease susceptibility (Vernerova *et al.*, 2014). Despite that, genetics is not an issue considered in current BSE prevention programs. In the case of CWD, it affects several species of cervids, so the genetic susceptibility or resistance will depend on each one. Studies have been conducted on some species, but no substantial results have been observed (Mead *et al.*, 2019).

Transmission routes

Although there are some differences among these diseases, there is a consensus that the infection occurs

mainly by oral route. Here, we describe aspects related to the transmission of the most relevant animal prion diseases (the natural transmission routes are summarized in Table 1).

Scrapie

The transmissible nature of scrapie has been proposed since the 1800s. However, it was associated with a parasite of the genus *Sarcosporidium*. It was not until the 1930s when the transmissibility of the causative agent was demonstrated experimentally, even though at that time it was believed to be a “filterable agent” (i.e., a virus) (Liberski, 2012).

Scrapie affects sheep, goats, and mouflons in natural conditions. But infection has been experimentally accomplished in rats (Chandler and Fisher, 1963), mice (Chandler, 1961), hamsters (Zlotnik and Rennie, 1965), and other species.

Horizontal transmission of scrapie

The exact natural route of infection is uncertain, but it is agreed that there is horizontal transmission (Greig, 1940). Following the oral route, it was observed that after entering, PrP^{Sc} is deposited in lymphoid tissue, such as Peyer's patches, mesenteric lymph nodes, and gut-associated lymphoid tissue (GALT) (Andreoletti *et al.*, 2000). From here, the agent spreads to the enteric nervous system, part of the PNS (McBride *et al.*, 2001; Heggebo *et al.*, 2003) (Fig. 1).

For the infection, PrP^{Sc} must pass through the epithelium of the gastrointestinal tract to reach the lymphoid tissue, where it is found in the early stages of the disease. Several mechanisms have been proposed, and the most accepted includes transcytosis by M cells (Heppner *et al.*, 2001; Miyazawa *et al.*, 2010) and possibly capture and transport by migratory dendritic cells (Huang *et al.*, 2002; Huang and MacPherson, 2004). At a cellular level, follicular dendritic cells (FDC) appear to play an essential role in scrapie pathogenesis. These cells are present in germinal centers of lymphoid

follicles and express quite quantities of PrP^C in normal conditions (McBride *et al.*, 1992). The presence of mature FDC is essential for the development of the disease. Scrapie infection was ineffective in mice with mature FDC deficiency (Brown *et al.*, 1999; Mabbott *et al.*, 2000). B lymphocytes are also important, but probably because they are necessary for the maturation and maintenance of FDC (Bruce *et al.*, 2000; Prinz *et al.*, 2003). B lymphocytes may also be involved in the first stage of spread from the earliest accumulation sites to secondary lymphoid organs such as the spleen via blood and lymph (Edwards *et al.*, 2010; Mok *et al.*, 2012). Macrophages are other cells where PrP^{Sc} can be found early, especially tingible body macrophages (TBM). These cells are present in the germinal centers of lymphoid nodules, and their function is to phagocyte apoptotic lymphocytes and, in this case, prions (Heggebo *et al.*, 2002; Herrmann *et al.*, 2003).

The nasal cavity was also proposed as a portal for PrP^{Sc} entry in horizontal transmission. This route of infection was confirmed by instilling a homogenate of the scrapie-infected brain into the nostrils of sheep that later developed scrapie (Hamir *et al.*, 2008). However, there are discrepancies in how the agent accesses the CNS. In a study, authors suggested that the olfactory system is involved in the natural transmission of scrapie based on PrP^{Sc} deposition in the olfactory bulb and olfactory cortex of naturally infected sheep (Corona *et al.*, 2009). On the other hand, assays in hamsters intranasally inoculated with PrP^{Sc} indicate that neuroinvasion occurs through unrelated olfactory pathways (Sbriccoli *et al.*, 2009). Beyond the discrepancy, the nasal route could be considered a route of entry in case of PrP^{Sc} was present in contaminated forage, bed, or soil and could be inhaled by sheep and goats.

Transepithelial transmission of scrapie has been studied since 1982, when infection through mucous membranes was first reported via gingival scarification in mice

Table 1. Summary of natural transmission routes in animal prion diseases.

Natural transmission route	Prion disease	References
Ingestion of prion contaminated food	Classic BSE	Anderson <i>et al.</i> , 1996
	TME	Marsh & Hadlow, 1992
	FSE	Baron <i>et al.</i> , 1999; Eiden <i>et al.</i> , 2010
	EUE	Kirkwood & Cunningham, 1994
	NHP prion disease	Bons <i>et al.</i> , 1996, 1999
Horizontal	Classic scrapie	Greig, 1940
	CWD	Miller & Williams, 2003
Vertical	Classic scrapie	Foster <i>et al.</i> , 1992, 1996; Spiropoulos <i>et al.</i> , 2014
	CWD	Nalls <i>et al.</i> , 2013, 2017; Selariu <i>et al.</i> , 2015

BSE: bovine spongiform encephalopathy; TME: transmissible mink encephalopathy; FSE: feline spongiform encephalopathy; EUE: exotic ungulate spongiform encephalopathy; NHP: nonhuman primate; CWD: chronic wasting disease.

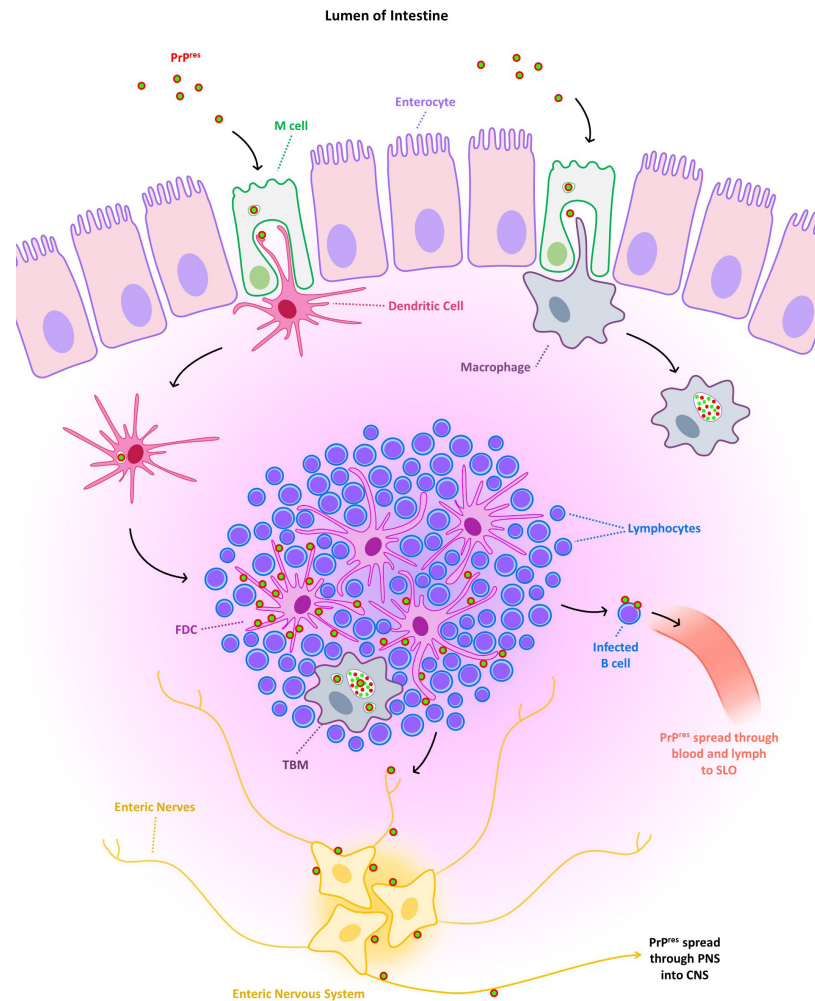


Fig. 1. Graphic representation of the possible spread of PrP^{res} from the lumen of the intestine following oral route. TBM: tingible body macrophage; FDC: follicular dendritic cell; SLO: secondary lymphoid organs; PNS: peripheral nervous system; CNS: central nervous system.

(Carp, 1982) and later percutaneously (Taylor *et al.*, 1996). In the following years, several investigations tried to understand the mechanisms of transcutaneous infection and how prion neuroinvasion occurred by this route (Mohan *et al.*, 2004, 2005). Although this form of transmission is possible, it does not seem as probable as the other proposed routes.

PrP^{Sc} has been detected in semen from scrapie-infected rams, and its infectivity has been proven in ovine transgenic mice (Rubenstein *et al.*, 2012). However, there is no evidence that scrapie can be transmitted by natural service or artificial insemination.

Sources of environmental contamination

As with other transmissible diseases, sources of environmental contamination could be involved in the spread of scrapie (Table 2). Secretions and

excretions of affected individuals may contribute to horizontal transmission. PrP^{Sc} was detected in feces of sheep naturally infected with scrapie in preclinical and clinical stages (Terry *et al.*, 2011). Studies done in Syrian hamsters suggest that it is possible to transmit scrapie through contaminated bedding of experimentally infected animals (Safar *et al.*, 2008). In the first days after oral infection, the detection of PrP^{Sc} could be due to the passage of the inoculum through the gastrointestinal tract but, once replicated in GALT and Peyer's patches, PrP^{Sc} could be detected in low quantities in the feces of the hamsters (Krüger *et al.*, 2009). These results could be influenced by the coprophagy that characterizes Syrian hamsters, which does not occur in the species naturally susceptible to scrapie. On the other hand, several studies have studied

Table 2. Summary of possible sources of infection in the transmission of scrapie in sheep and goats.

Source of infection	Methods of detection	References
Placental tissue	Transmission to sheep, goats, and mice; detection of PrP ^{Sc} by immunoblotting, IHC, and ELISA	Andréoletti <i>et al.</i> , 2002; Lacroux <i>et al.</i> , 2007; O'Rourke <i>et al.</i> , 2011; Onodera <i>et al.</i> , 1993; Pattison <i>et al.</i> , 1972, 1974; R. E. Race <i>et al.</i> , 1998; Tuo <i>et al.</i> , 2002
Milk and colostrum	Transmission to sheep, goats, and ovine transgenic mice; detection of PrP ^{Sc} by PMCA	Konold <i>et al.</i> , 2008, 2013, 2016; Lacroux <i>et al.</i> , 2008; Maddison <i>et al.</i> , 2009; Zhuang <i>et al.</i> , 2018
Feces	Detection of PrP ^{Sc} by PMCA	Terry <i>et al.</i> , 2011
Urine	Detection of PrP ^{Sc} by PMCA	Rubenstein <i>et al.</i> , 2011
Saliva	Transmission to ovine transgenic mice and detection of PrP ^{Sc} by PMCA	Gough <i>et al.</i> , 2012; Maddison, <i>et al.</i> , 2010; Tamgüney <i>et al.</i> , 2012
Semen	Transmission to ovine transgenic mice and detection of PrP ^{Sc} by PMCA	Rubenstein <i>et al.</i> , 2012
Blood	Transmission to sheep and detection of PrP ^{Sc} by PMCA	Houston <i>et al.</i> , 2008; Thorne & Terry, 2008

IHC: immunohistochemistry; ELISA: enzyme linked immunosorbent assay; PMCA: protein misfolding cyclic amplification

urine as a source of infection. In assays performed in mice and hamsters, it was demonstrated the infectivity and the presence of PrP^{Sc} in the urine of rodents infected with scrapie (Seeger, 2005; Kariv-Inbal *et al.*, 2006; Murayama *et al.*, 2007; Gonzalez-Romero *et al.*, 2008; Gregori *et al.*, 2008). In the case of species naturally affected by scrapie, PrP^{Sc} has also been detected in the urine of infected sheep by using PMCA (Rubenstein *et al.*, 2011). Therefore, excretions of infected animals might be a risk to others.

Regarding secretions, saliva could play a role in horizontal transmission in two ways: environmental contamination and direct contact between animals. PrP^{Sc} was detected in oral secretions of sheep infected with scrapie in preclinical and clinical stages (Maddison, *et al.*, 2010; Tamgüney *et al.*, 2012). Infectivity could also be verified in transgenic mice expressing ovine PrP^C (Gough *et al.*, 2012; Tamgüney *et al.*, 2012). In addition, it was suggested that when infected animals ingest the saliva contaminated with PrP^{Sc}, it may reinfect gastrointestinal tissues. This reinfection could contribute to eliminating the faecal prions since the PrP^{Sc} detected in saliva was similar to the PrP^{Sc} detected in the feces of scrapie-infected sheep (Tamgüney *et al.*, 2012).

The blood of animals naturally infected with scrapie was infectious in both preclinical and clinical stages. Transmissibility in sheep has been demonstrated through transfusion of whole blood and buffy coat from scrapie-infected donors to healthy recipients (Houston *et al.*, 2008). Furthermore, the presence of PrP^{Sc} could be detected in the cell fractions of the blood of naturally infected sheep (Thorne and Terry, 2008).

Blood transfusion is not a common practice in sheep and goats, but it is frequent in humans, and CJD could be a health hazard for the recipient.

In addition to the contamination of the environment, secretions, or excretion can also contaminate inanimate objects, and these act as indirect transmitters of scrapie. For example, it was confirmed transmission through field furniture that had been in contact with an infected scrapie flock, indicating that these objects may act as a reservoir for PrP^{Sc} (Konold *et al.*, 2015).

Vertical transmission of scrapie

Vertical transmission seems to be another way of maintaining scrapie in goat and sheep herds. There is a higher incidence of scrapie in offspring of naturally affected ewes (Hoinville *et al.*, 2010) although this could be for several transmission ways. The higher incidence could be explained by the transmission through milk or colostrum. PrP^{Sc} has been detected in sheep's milk infected with scrapie, even in preclinical stages of the disease (Lacroux *et al.*, 2008; Maddison *et al.*, 2009). Furthermore, it was possible to transmit scrapie to lambs using sheep and goat milk or colostrum from infected animals (Konold *et al.*, 2008, 2013, 2016). This fact was also confirmed in goats in recent studies since scrapie was transmitted to lambs and kids using milk from goats clinically and preclinically affected with scrapie (Zhuang *et al.*, 2018).

Although some studies have shown that embryo transfer is a safe method to prevent scrapie infection from mothers to lambs (Foote *et al.*, 1993; Wang *et al.*, 2001; Low *et al.*, 2009), other studies have reported the possibility of transmission of scrapie *in utero* (Foster

et al., 1992, 1996; Spiropoulos *et al.*, 2014). It was observed that experimentally infected ewes with a susceptible genotype could infect their offspring, and infection could not be prevented by embryo transfers or cesarean section and immediate separation from their mothers (Foster *et al.*, 2013). Therefore, the genotype could play a role in the transmission *in utero*.

Infectivity of placental tissues was confirmed by several reports. In the 1970s, scrapie transmission to sheep had already been proven through oral administration of fetal membranes (Pattison *et al.*, 1972, 1974), even before the causative agent was known. It was not until the 1990s when PrP^{Sc} was detected in placental tissues of sheep by immunoblotting (Race *et al.*, 1998). Subsequent studies evidenced that the accumulation of PrP^{Sc} depends on the *PRNP* genotype of the fetus. Scrapie-infected ewes can accumulate large amounts of PrP^{Sc} in placentomas only if the fetus possesses a genotype susceptible to contracting the disease (Andréoletti *et al.*, 2002; Tuo *et al.*, 2002; Lacroux *et al.*, 2007; Garza *et al.*, 2017). This discovery was essential for the development of sheep breeding programs that were implemented in different countries. In the case of goats with naturally acquired scrapie, the accumulation of PrP^{Sc} in placental tissues is low (O'Rourke *et al.*, 2011). Despite the slight amount of PrP^{Sc}, goat placental tissues maintain their infective potential, and it has been possible to transmit scrapie to lambs and kids administering infected placenta orally (Schneider *et al.*, 2015). These studies reveal that placental tissues are an important source of infection that could contaminate the environment in the lambing season.

Several authors indicate that PrP^{Sc} accumulation increases in the presence of chronic inflammation in certain tissues. Considering transmission, this fact becomes relevant when chronic inflammation occurs in organs related to excretion or secretion. Some authors observed that scrapie transmission through milk can be enhanced by chronic lympho-follicular mastitis in sheep with lentiviral coinfection (Lacroux *et al.*, 2008; Ligios *et al.*, 2011). This enhancement is probably resulting from the action of small ruminant lentiviruses (SRLV). They produce an inflammatory reaction that favors the accumulation of PrP^{Sc} at the site of inflammation (Salazar *et al.*, 2010). Increased prion aggregation also occurs in the mammary glands of goats affected with scrapie and coinfecting with SRLV (González *et al.*, 2010; Zhuang *et al.*, 2018). This finding is important since SRLV infections are highly prevalent and global endemic, and they could act as enhancers in the transmission and perpetuation of scrapie in sheep and goat flocks.

Bovine spongiform encephalopathy

The first case of classical BSE was reported in 1986 (Wells *et al.*, 1987), and 2 years later, its transmissibility could be verified through inoculation in mice (Fraser *et al.*, 1988). Although the origin of the outbreak remains uncertain, the transmission of BSE has been well studied in subsequent years due to the risk of transmission to humans. Many epidemiological studies were conducted to understand this novel disease. Thus, the incidence of

BSE was found to be higher in dairy than in beef herds, and it could result from differences in feeding modes of both production systems (Bradley, 1991; Wilesmith *et al.*, 1992). Based on these epidemiological data and the absence of direct horizontal transmission, it was established that the primary source of BSE infection was the food containing meat and bone meal with which the cattle were fed (Anderson *et al.*, 1996). This fact led to the prohibition of feeding cattle with ruminant proteins in several countries.

The pathogenesis of BSE, following the oral route, is similar to scrapie in the early stages. PrP^{BSE} is initially deposited in Peyer's patches, GALT, and tonsils, and then it is distributed to the CNS through the PNS (Terry *et al.*, 2003; Wells *et al.*, 2005; Hoffmann *et al.*, 2007, 2011). The main difference with scrapie is that individuals affected with BSE show slight or no distribution of prions in lymphoid tissue, except for those already mentioned (Birkett *et al.*, 1997; Wells *et al.*, 1998; Espinosa *et al.*, 2007).

Due to new cases of BSE in animals born after the feed ban in the United Kingdom (UK), some researchers suspected that vertical transmission was also possible (Lacey and Dealler, 1994; Curnow and Hau, 1996). Some cohort studies indicated an increased risk of developing BSE in calves born from dams that had already developed BSE clinical signs (Donnelly *et al.*, 1997; Wilesmith *et al.*, 1997). Years later, vertical transmission of BSE was achieved in a transgenic mouse model (Castilla *et al.*, 2005). However, there is no clear evidence of vertical transmission in cattle. Also, it was shown that embryos would not be infectious even if they were collected from dams in the final stage of BSE (Wrathall *et al.*, 2002). In fact, several authors proposed that the cause of the new cases of BSE in animals born after the feed ban was the cross-contamination with feed for other production animals such as pigs or poultry, which could legally contain meat and bone meal (Abrial *et al.*, 2004; Stevenson *et al.*, 2005; Pottgiesser *et al.*, 2006; Allepuz *et al.*, 2007; Jarrige *et al.*, 2007; Paul *et al.*, 2007; Schwermer *et al.*, 2007).

Milk as a source of PrP^{BSE} was investigated because it could be a route of transmission between dam and calf, but mainly because of the risk to humans related to the consumption of dairy products. Many studies have been done on this subject, but to date, there is no evidence that BSE can be transmitted through milk or colostrum (Domingo, 2002; Vetrugno, 2004; Everest *et al.*, 2006). Regarding other secretions, PrP^{BSE} has been detected in concentrated samples of the saliva of cattle affected with BSE (Okada *et al.*, 2012) although its infectivity was not established. More studies would be needed to expand the knowledge on the potential infectivity in secretions or excretions of BSE-infected animals, but the current low incidence of the disease might suggest that saliva is not an essential source for the infection.

BSE in small ruminants

As described, research on BSE indicated that feed was the main route of transmission of the disease. Small ruminants had been exposed to the same feed

as cattle during the 1980s. Some investigations were carried out to discern the transmissibility of BSE in small ruminants and to determine the risk of the spread. It was shown that sheep and goats are susceptible to BSE by the oral or intracranial route and also that clinical signs produced by BSE remain indistinguishable from scrapie (Foster *et al.*, 1993; Van Keulen *et al.*, 2008). Therefore, the possibility that PrP^{BSE} had been transmitted to sheep and goats constitutes another potential risk to public health. The distribution of PrP^{BSE} in the tissues of experimentally infected sheep was similar to those observed in scrapie with a wide distribution in lymphoreticular tissue (Jeffrey *et al.*, 2001; Bellworthy *et al.*, 2005). It would be difficult to control if BSE in small ruminants were transmitted by the same routes in which scrapie is spread. Furthermore, scrapie-resistant genotypes are susceptible to contracting BSE. Thus, the applied breeding programs would not be helpful in preventing the spread of BSE in sheep herds (Foster *et al.*, 1993; Jeffrey *et al.*, 2001).

Despite all this concern, only two cases have been detected in goats with a natural prion disease indistinguishable from BSE in experimentally infected goats (Eloit *et al.*, 2005; Jeffrey *et al.*, 2006). On the other hand, no case linked to BSE has been reported in sheep.

Maternal or vertical transmission of BSE was demonstrated in 18% of lambs born from sheep experimentally infected (Jeffrey *et al.*, 2015). The horizontal transmission was also observed in 0.5% of cases, although this value is lower than expected with scrapie. This poor transmission is probably due to the low presence of PrP^{BSE} in placental tissues (Jeffrey *et al.*, 2015). Despite these findings, the deficient transmission observed both vertically and horizontally suggests that it is unlikely that BSE could be maintained within a herd of sheep.

Chronic wasting disease

As previously mentioned, CWD of cervids is considered as a concern because it is maintained in wildlife animals and has spread across large areas.

CWD has been known since the late 1960s as a syndrome affecting mule deer (*Odocoileus hemionus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) in captivity. In 1980, CWD was clinically and pathologically characterized as a spongiform encephalopathy (Williams and Young, 1980). Over the years, CWD was also described in several species of captive and free-ranging cervids such as Rocky Mountain elk (*Cervus elaphus nelsoni*), white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), elk (*Cervus canadensis*), red deer (*Cervus elaphus elaphus*), sika deer (*Cervus nippon*), and reindeer (*Rangifer tarandus*) (Schwabenlander *et al.*, 2013; Benestad *et al.*, 2016).

Based on other animal prion diseases, the main route of entry for PrP^{CWD} is presumed to be oral, which has been experimentally achieved in various species of

cervids (Sigurdson *et al.*, 1999; Kreeger *et al.*, 2006; Balachandran *et al.*, 2010; Mitchell *et al.*, 2012). The pathogenesis through ingestion and tissue distribution of PrP^{CWD} seems similar to that described in scrapie-infected sheep. There is an early distribution in lymphoid tissue, followed by spread to the CNS, PNS, and other organs (Sigurdson *et al.*, 1999, 2001, 2002; Fox *et al.*, 2006). But unlike what was observed in scrapie-infected sheep, PrP^{CWD} accumulation was greater in retropharyngeal lymph nodes than in Peyer's patches of CWD-infected deer (Sigurdson *et al.*, 1999; Fox *et al.*, 2006).

Similar to what happens in scrapie, direct, or indirect horizontal transmission has been observed in CWD (Miller and Williams, 2003), indicating that lymphoid tissue has a role in the transmissibility of the disease. In a study comparing PrP^{CWD} levels between deer and elk affected with CWD, the distribution of prions in extraneural tissues was lower for elk (Race *et al.*, 2007). The authors suggest that this low extraneural peripheral distribution of PrP^{CWD} could explain two situations: first, the low incidence of CWD in elk; and second, insufficient horizontal transmission of CWD in elk compared to deer.

In 2004, it was demonstrated that susceptible animals (mule deer) could contract CWD after the exposition to contaminated environments or infected animals (Miller *et al.*, 2004). From this investigation, saliva, urine, and feces have been studied as possible sources of natural infection. The infectivity of saliva from infected animals was confirmed by oral administration to healthy deer that developed CWD after exposure (Mathiason *et al.*, 2006, 2009). However, the infectivity of urine and feces was not confirmed in this assay. Years later, PrP^{CWD} was detected in saliva, urine, and feces of affected animals, and their infectivity was demonstrated in the mice model (Haley *et al.*, 2009; Pulford *et al.*, 2012). Additionally, PrP^{CWD} could even be detected in saliva (Henderson *et al.*, 2013), urine (John *et al.*, 2013), and feces (Tamgüney *et al.*, 2009) of asymptomatic animals. These findings suggest that infected but healthy animals could be a source for infection of other animals. On the other hand, detection of PrP^{CWD} in saliva, urine, and feces could be used as an alternative, non-invasive and preclinical diagnosis of animals.

Since the numerous similarities between CWD and scrapie, several studies were conducted to investigate the possibility of vertical transmission in CWD. The first evidence of transmissibility between fawns and infected does was carried out on Reeves' muntjac deer, a species experimentally susceptible to the disease but with no reports of natural transmission to date (Nalls *et al.*, 2013). This study demonstrated the presence of PrP^{CWD} in harvested fetal tissues from infected pregnant does. In a subsequent investigation using the same cervid model, infective prions were detected in placentomes and amniotic fluid (Nalls *et al.*, 2017).

PrP^{CWD} accumulation could also be evidenced in the female reproductive tract and fetal tissues of naturally exposed free-ranging elks (Selariu *et al.*, 2015). All these findings suggest that transmission *in utero* of CWD is possible and that placental fluids and tissues could be another source of environmental contamination.

Atypical prion diseases

In 1998, some cases of scrapie were reported in Norway. These cases showed different clinical signs than typical scrapie (i.e., pruritus was absent), and the pathologic changes were unusually distributed (Benestad *et al.*, 2003; Nentwig *et al.*, 2007; Moore *et al.*, 2008; Cook *et al.*, 2016). PrP^{Sc} isolated from atypical scrapie cases also vary in the biochemical characteristics such as glycosylation and cleavage sites (Benestad *et al.*, 2008). Regarding genetic susceptibility, atypical scrapie can affect sheep with genotypes resistant to the classical form (Buschmann *et al.*, 2004; De Bosschere *et al.*, 2007).

In 2004, cases of BSE detected in Italy and France showed differences from the typical cases. The isolated prions showed fragments of peptides with different molecular masses after digestion with proteases. The one with fragments higher than previous cases of BSE was called H-BSE; the other with a fragment with lower molecular mass was called L-BSE. Then, the typical form of disease began to be called classical BSE (C-BSE), and H and L-BSE are recognized as “atypical forms of BSE.” Since its low prevalence, it is challenging to investigate atypical cases of BSE in its natural state, but the disease has been reproduced experimentally.

Atypical scrapie and atypical BSE generally occur in elderly animals and have a sporadic distribution and low prevalence, unlike the classic forms of these diseases (Brown *et al.*, 2006; Fediaevsky *et al.*, 2008; Sala *et al.*, 2012). In addition, atypical cases have been reported in countries or regions where classical prion diseases are exotic diseases, such as H-BSE cases in Norway, Sweden, and Brazil (OIE, 2020) or atypical scrapie cases in New Zealand (Kittelberger *et al.*, 2010), Australia (Cook *et al.*, 2016) and the Malvinas' (Falkland) Islands (Epstein *et al.*, 2005). These findings suggest that the origin of the atypical form of prion diseases is completely different from the origin of the classical form.

Although the first reported case of atypical scrapie was in 1998, retrospective studies showed that atypical cases have existed since at least 1987 (Webb *et al.*, 2009). The potential transmissibility of atypical scrapie was experimentally verified intracranially and orally in sheep (Simmons *et al.*, 2007, 2011) and ovine transgenic mice (Le Dur *et al.*, 2005). Nevertheless, there is no evidence that atypical scrapie is transmitted under natural conditions. The absence of horizontal transmission could be related to the lack of distribution and accumulation of PrP^{Sc} in the peripheral tissues of animals affected with atypical scrapie (Benestad *et al.*, 2003; Buschmann *et al.*, 2004; Nentwig *et al.*,

2007). However, one study demonstrated that in natural and experimental cases of atypical scrapie in sheep, peripheral tissues can be infective for transgenic mice even though PrP^{Sc} is not detected through classical techniques such as immunohistochemistry, enzyme immunoassay, or immunoblot (Andréoletti *et al.*, 2011). PrP^{Sc} could be present in small quantities, probably under the analytic sensitivity of those techniques. In that case, the epidemiologic impact of instances of atypical scrapie should be evaluated in further studies. In the case of atypical BSE, both H-type and L-type infectivity could be demonstrated in transgenic mice overexpressing bovine PrP^C (Buschmann *et al.*, 2006), and then via intracranial in cattle (Lombardi *et al.*, 2008; Balkema-Buschmann *et al.*, 2011). The infectivity of peripheral nervous tissue and skeletal muscle has been demonstrated in cattle experimentally infected with L-BSE and H-BSE (Sawada *et al.*, 2019). However, the oral route would not be an efficient route of transmission based on the results in cattle challenged with L-BSE (Okada *et al.*, 2017). Only 1 of 16 orally inoculated calves developed mild clinical signs after 88 months of incubation, and this cow had been inoculated with a high dose of L-BSE infected brain homogenate. Based on these results and the epidemiological data, it is believed that the atypical forms of prion diseases may have a spontaneous origin and their transmission could be very low or null in natural conditions.

Other animal prion diseases

In addition to the aforementioned species, prion diseases have been reported in minks, felids, several species of antelopes, and nonhuman primates (Hartsough and Burger, 1965; Bons *et al.*, 1996, 1999; DEFRA, 2019). These reports are believed to be the result of ingestion of food contaminated with ruminant prions. TME was associated with scrapie and more recently with L-BSE (Hanson *et al.*, 1971; Comoy *et al.*, 2013). Reports of prion diseases in felids, nonhuman primates, and antelopes appeared from 1990, mainly in the UK during the BSE outbreak, and were associated with the consumption of PrP^{BSE} (Jeffrey *et al.*, 1992; Kirkwood and Cunningham, 1994; Baron *et al.*, 1999; Eiden *et al.*, 2010). In fact, after the measures are taken to control BSE, no more cases of prion diseases were reported in the mentioned species.

Novel prion disease was recently reported in dromedary camels (*Camelus dromedarius*) from Algeria, designated as CPD (Babelhadj *et al.*, 2018). Three affected animals showed neurological signs during a routine ante-mortem inspection in a slaughterhouse. Then, the presence of PrP^{CPD} was detected in nervous tissues by immunohistochemistry and immunoblotting along with the characteristic spongiform lesions of prion disease. PrP^{CPD} was also detected in peripheral lymph nodes in one of the affected animals. This is important since the distribution of prions in peripheral lymphoid tissue is associated with horizontal transmission in scrapie and

CWD. More studies should be developed to advance the knowledge of this disease.

Conclusions

Since scrapie was described in 1732, knowledge of animal prion diseases has increased, particularly after the prion hypothesis was proposed. The appearance of BSE in the late 1980s and its subsequent association with the variant of CJD put prion diseases in the spotlight. The transmission route and infection sources of prion diseases were studied in depth to take epidemiological measures to control them.

Direct and indirect horizontal transmission has been observed in scrapie and CWD due to the excretion of prions into the environment. In both diseases, PrP^{res} can be detected in non-nervous tissues. The extraneural distribution of PrP^{res} appears to be necessary for horizontal transmission of animal prion diseases.

Besides BSE, there are currently no other prion diseases considered a risk to Public Health. However, the wide distribution of CWD, the transmission of scrapie to humanized mouse and the recently described CPD are issues to consider. Thus, further research is important to understanding the potential risks of prion diseases to other animals or humans.

Conflict of interest

The authors declare that there is no conflict of interest.

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