

A REVIEW: BOVINE SPONGIFORM ENCEPHALOPATHY ASSOCIATED WITH PRNP GENE POLYMORPHISMS

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Abstract

Bovine Spongiform Encephalopathy (BSE) is a chronic, degenerative disease that affects the central nervous system of cattle, the condition being known as "mad cow disease." BSE is part of the family of transmissible spongiform encephalopathies (TSEs). The main characteristics of TSEs refer to: a) very long incubation period, months or even years; b) progressive neurological disease, often fatal; (c) brain tissue from infected animals showed fibrils associated with scrapie; (d) pathological changes occur only in the central nervous system. Another disease in the EST category is scrapie, which was initially thought to be specific to sheep and does not affect humans, although it was known to be an infectious agent. As there was no other known spongiform encephalopathy at the time of the onset of BSE, it was considered to be derived from scraps, especially given that sheep meat was often served to cows to increase milk production.

Keywords: BSE; cattle; TSE, prions, CJD.

INTRODUCTION

Bovine spongiform encephalopathy (BSE) belongs to the group of transmissible spongiform encephalopathies (TSEs), also known as prion diseases, which are fatal protein-misfolding neurodegenerative diseases. Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative diseases that can occur spontaneously or can be caused by infection or mutations within the prion protein gene PRNP (Kashkevich K. et al., 2007). Transmissible spongiform encephalopathy (TSE) agents or prions induce fatal neurodegenerative diseases in humans and in other mammals.

They are transmissible among their species of origin, but they can also cross some species barriers and induce infection with or without disease in other species. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker syndrome, Kuru, and fatal familial insomnia. In animals, 4 distinct TSE diseases are recognized: scrapie in sheep and goats, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. BSE is transmissible via BSE-contaminated feed to felines (feline spongiform encephalopathy, FSE) and exotic ungulates (exotic ungulate encephalopathy, EUE) (Richt J.A

et al., 2007). These chronic diseases are associated with the accumulation of a protease-resistant (Eraña H. et al., 2020) disease associated isoform of the prion protein (PrP) in the central nervous system and other tissues, depending on the host species (Greenlee Justin J.J et al., 2012). The prion protein (PrP) is encoded by PRNP gene, in brain and other tissues. Although the exact function of this infectious protein is unknown, they are derived from natural cellular proteins (PrPc), which are thought to play a role in natural synaptic function (Collinge J. et al., 1994) or in the outgrowth and survival of neurites (Chen S. et al., 1994). The physiological PrPc will shift the pathological PrPsc (scrapie like prion proteins), which is contagious, to its normal conformation (Kim M.O. et al., 2018). The abnormal types of prion protein (PrPsc) fold into insoluble amyloid, are extremely resistant to protease metabolism, and can cause normal prion (PrPc) conformational shift. Therefore, if a mutation in the prion gene contributes to the development of PrPsc, a prion disorder may be hereditary and it can be contagious if exposure to PrPsc causes the PrPc of the host to undergo conformational shift (MacKnight C., 2001).

Several known forms of PrP have been identified, but is known that prion protein (PrP) plays a central role in the pathogenesis of

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neurodegenerative diseases such as the bovine spongiform encephalopathies (BSE) in cattle, scrapie in sheep, and Creutzfeldt–Jakob disease in humans (Prusiner S.B., 1989). PrP are practically host-coded proteins that have undergone conformational changes and have biological and physicochemical characteristics that differ significantly from those of other infectious agents. For example, they are resistant to inactivation processes that are effective against conventional viruses including those that alter nucleic acid structure or function according to Prusiner's generally accepted hypothesis.

RESULTS AND DISCUSSIONS

Typically, TSEs are acquired through exposure to infectious material, but inherited and spontaneous TSEs also occur. All TSEs share pathologic features and infectious mechanisms but have distinct differences in transmission and epidemiology due to the host factors and strain differences encoded within the structure of the misfolded prion protein. So that, the causative agent of TSEs is an infectious prion protein (PrP^{Sc}), that is a structurally abnormal and protease resistant isoform of the prion protein (PrP) (Belay E.D., 1999). PrP^{Sc} is derived from the endogenous cellular prion protein (PrP^C) that has undergone post-translational modification resulting in conformational changes. PrP^C is known to play a role in copper metabolism but its normal function(s) in cells are not well defined (Choi S. et al., 2011). Practically, in TSE diseases, the normal cellular protein, PrP^C, is converted to abnormal prion protein, PrP^{Sc}, which exhibits increased beta sheet content, a change that may drive the additional changes in solubility and protease resistance. Unlike normal cellular protein, PrP^{Sc} is relatively insoluble in detergents, is relatively resistant to proteases and is capable of causing a conformational change in additional molecules of PrP^C (Richt J.A. et al., 2007). The incidence of TSEs, also referred to as prion diseases, in a defined host population is influenced by a variety of factors. In the individual animal, however, the disease is always associated with an increase of the protease-resistant form of the cellular prion protein, which is then denoted scrapie-associated prion protein (PrP^{Sc}) (Prusiner S.B., 1998). In cattle, BSE in cattle is usually thought to be caused by the ingestion of meat and bone meal from scrapie-infected sheep or cattle infected with BSE. The ingestion of meat and bone meal (MBM) produced from scrapie-infected sheep or from cattle with BSE represents the most likely cause of the large BSE outbreak in cattle in the United

Kingdom (Eddy R.G., 1995). The function of the physiological prion protein isoform (PrP^C) has not yet been elucidated. Affected animals display changes in temperament, abnormal posture, incoordination and difficulty in rising, decreased milk production, and/or loss of body weight despite continued appetite. BSE-affected cattle undergo progressive nervous system degeneration. Animals affected can exhibit changes in temperament, posture and movement abnormalities, and changes in sensation. More precisely, there are signs of anxiety, nervousness or aggression, incoordination, especially hind-limb ataxia, tremor and rising difficulty, and sound and touch hyperesthesia. Additionally, despite continued appetite, many animals have reduced milk production, loss of body condition, or both. There is no cure, and the cattle affected die. The time of incubation varies from 2 to 8 years. The animal's condition gradually deteriorates after the onset of clinical symptoms, until the animal becomes recumbent, dies, or is killed. Usually, this takes between 2 weeks and 6 months (Detwiler L.A. et al., 2000). The majority of cases in Great Britain occurred between the ages of 3 and 6 years in Holstein Frisian dairy cows (Wilesmith J.W. et al., 1992).

It has been proposed that PrP^C plays a role in normal synaptic function (Collinge J. et al., 1994) or in cell-cell interactions and acts as an anti-apoptotic signaling molecule (Premzl M. et al., 2007). BSE was first diagnosed among Holstein/Friesian cattle in the United Kingdom (Wells G.A. et al., 1987) and has since been detected in other countries as well.

From the nearly first cases, the brains of two cows exhibiting neurological symptoms were examined at the United Kingdom Central Veterinary Laboratory in November 1986 by two neuropathologists who noted lesions similar to those usually found in scrapie-affected sheep brains, i.e. spongiform tissue changes (Wells G.A. et al., 1987). Some British researchers have, after collecting epidemiological data, linked the possible cause of the disease to certain animal proteins found in bovine feed. The disorder was diagnosed as a prion disease and called bovine spongiform encephalopathy (BSE) due to its resemblance to scrapie and unusual histopathological symptoms, spongiform tissue changes. As the new variant CJD (vCJD) in humans is likely the result of infection with BSE prions found in meat products from BSE-infected cattle, BSE poses a threat not only to cattle but also to humans (Bruce M.E. et al., 1997; Scott M.R. et al., 1999; Asante E.A et al., 2002).

Also, in some TSE, there is also potential for horizontal transmission, which simply means transmission directly from one animal to an adjacent animal in the herd. However, unlike scrapie in sheep and CWD in deer where horizontal transmission has been shown (Gough K.C. et al., 2010), there is no evidence of horizontal transmission of BSE in cattle. Another proposed route of disease exposure is termed vertical transmission, which is best explained as transmission mainly from parents to offspring either in utero or through birth or lactation. While the possibility of vertical transmission has not been entirely excluded in cattle, it is considered to be very low in incidence if at all, and there is no evidence of prion transmission in milk (Everest S.J. et al., 2006) by ELISA detection, embryos or semen (Wrathall A.E. et al., 2002) via histopathology, immunohistochemistry, or bioassay detection.

Until 2004, TSE disease in cattle was believed to be caused by a single prion strain, classical BSE (BSE-C or cBSE). This conclusion was based on classical strain typing in mice (incubation time, lesion profiles, patterns of PrP staining in the brain) and biochemical features of the proteinase K (PK) resistant PrP (PrPTSE) in natural and experimental BSE, which showed consistent results from all cattle isolates. However, two atypical BSE agents have been reported in 2004 – H-BSE (Biacabe A.G. et al., 2004) and L-BSE (Casalone C. et al., 2004). These variants seem to be sporadic, and all occurred in animals 8 years and older (Langeveld J.P.M. et al., 2011) and can be distinguished from classical BSE by its pathological, molecular and biological phenotype (Konold T. et al., 2014) by the electrophoretic positions of their protease-resistant PrPTSE isoforms (Wilson R. et al., 2012). Indeed, PrP is a glycoprotein and has two sites for the attachment of N-linked glycans, which depending on their utilisation will produce di-, mono-, and unglycosylated PrP. BSE-H PrPTSE shows a significantly higher molecular weight unglycosylated PrP isoform by immunoblot when compared with BSE-C PrPTSE. Similarly, BASE has a slightly lower molecular size than BSE-C PrPTSE and a clearly different glycoform pattern. Furthermore, following transmission into transgenic mice that overexpress the bovine prion protein, both BASE and BSE-H show neuropathological and molecular phenotypes which are distinct from BSE-C (Béringue V. et al., 2007). However, interestingly a recent study has found that survival times are similar in transgenic mice that overexpress the bovine prion protein challenged

with either BSE-C or BSE-H (Torres J.M. et al., 2011). BSE-H and BASE were originally described in France (Biacabe A.G. et al., 2004) and Italy (Casalone C. et al., 2004) respectively, however have since been documented in other European countries (Jacobs J.G. et al., 2007), Japan (Hagiwara K. et al., 2017) and North America (Richt J.A. et al., 2007). While BSE-C is thought to be the result of feeding cattle prion-contaminated meat and bone meal, the origin of BASE and BSE-H remains unknown [28].

Resistance to cBSE in cattle has been found to be modulated by 2 nucleotide polymorphisms in regulatory regions of the prion gene (PRNP) (Sander P. et al., 2004; Jüling K. et al., 2006; Seabury C.M. et al., 2004). The first is an insertion-deletion (indel) in the promoter region, where the 23-bp deletion removes a binding site for the repressor protein RP58. The second polymorphism is an indel in the first intron, where the 12-bp deletion removes a binding site for transcription factor SP1. Insertion variants of either regulatory element have the potential to lower host prion protein expression levels (Sander P. et al., 2004), thus providing a biological basis for BSE resistance in cattle homozygous for the presence of the insertions. H-type BSE has been described in cattle from France (Biacabe A.G. et al., 2004), Germany (Buschmann A. et al., 2006), Japan (Sugiura K. et al., 2009), the Netherlands (Biacabe A.G. et al., 2007), Poland (Jacobs J.G. et al., 2007), Switzerland (Tester S. et al., 2009), the United Kingdom (Stack M. et al., 2009), Canada (Dudas S. et al., 2010), United States (Richt J.A. et al., 2007) and Sweden (Gavier-Widen D. et al., 2008). The molecular phenotype of the H-type BSE cases is characterized by a higher molecular mass of the unglycosylated PrP^{Sc} isoform and a strong labeling of all 3 PrP^{Sc} polypeptides.

There are some genetic variations of PRNP gene in cattle, so that several experiments have been carried out in cattle to find such a relationship between BSE and cattle genome polymorphisms (Goldmann W. et al., 1990; Neibergs H.L. et al., 1994; Heaton M.P. et al., 2004).

Genetic variations in the prion protein gene (PRNP) are linked to the occurrence of transmissible spongiform encephalopathies (TSEs) also called prion diseases in humans, sheep and mice. It has been reported that single nucleotide polymorphisms (SNPs) of PRNP have various effects on the susceptibility and incubation time of prion diseases in humans, mice, and sheep (Baylis M. et al., 2004). In sheep, polymorphisms in codons 136, 154, and 171 are correlated with susceptibility to scrapie and can

thus be used to control disease incidence (Hills D. et al., 2001) whereas in humans, a polymorphism in codon 129 has a critical influence to variant CJD incidence (Wadsworth J.D.F. et al., 2004).

The prion gene, *PRNP*, in cattle is located on the forward strand of chromosome 13, from 47,400,413 to 47,418,507 base pairs (bp), within syntenic group U11 (Ryan A.M. et al., 1993) and mapped on BTA13 chromosome (13q17) (Schlöpfer J. et al., 1999). This gene extends over 20.2 kb, and full-length mRNA containing three exons is 4244 bp. Exon 1 spans 53 bp and exon 2 spans 98 bp (Inoue S., et al., 1997). The size of the second intron has been estimated to be approximately 14 kb (Horiuchi M. et al., 1998). The whole ORF is located within exon 3 and has a size of 795 bp (Yoshimoto J. et al., 1992). The complete genomic sequence of 78 056 bp has also been determined and deposited in the EMBL/GenBank database under accession number AJ298878 (Hills D. et al., 2001). In cattle, the prion gene consists of three exons, as compared to the human gene which is composed of two exons. However, consistent in all orthologs across species, only the last exon is translated to a protein. There are three genes within this chromosomal locus, the prion gene *PRNP*, the doppel gene *PRND*, and the testis-specific alternatively spliced transcription product *PRNT* (Murdoch B.M. et al., 2015). The doppel gene *PRND*, also called prion-like gene (prion protein 2), is located immediately downstream (47,444,352–47,449,390 bp) of the *PRNP* gene. Interestingly, the doppel protein, despite its genomic proximity to the *PRNP* gene is not expressed at appreciable levels in the brain; however, it is expressed in the testis and in fact its absence in the testis results in sterility. A third member of this gene locus is prion protein testis-specific *PRNT* found immediately downstream to *PRND* but on the reverse strand. Expression of the *PRNT* gene is exclusively found in the adult testis in human, rhesus monkey, and sheep; however, it is not observed in mouse, rat, and cow (Premzl M. et al., 2007).

In addition to the three previously described genes, another gene with a role in prion disease has been discovered. Shadow of prion protein homolog and its gene *SPRN* has been mapped to the reverse strand of chromosome 26, from 25,812,626 to 25,813,057 bp in cattle. Importantly, in addition to the prion gene, *SPRN* has been implicated in prion-related disease susceptibility. Specifically, *SPRN* has been associated with prion disease in cattle (Uboldi C. et al., 2006; Gurgul A. et al., 2012) and humans (Beck J.A. et al., 2008).

SPRN is expressed in high levels in the brain and at lower levels in testis. Due to the fact that *SPRN* is more conserved than *PRNP*, it has been hypothesized that it is the ancestor gene of a duplication event. The model proposes that the duplication of *SPRN* gave rise to *SPRNB*, which then resulted in the *PRNP* gene cluster (Beck J.A. et al., 2008). While there is homology across these genes and some suspected redundancies, the full extent and significance of these relationships have yet to be fully characterized and, of course, merits further study. Again and somewhat unique to prion diseases, an animal cannot accumulate misfolded proteins, the hallmark of TSE disease, if they do not have that protein to begin with. Therefore, the native prion protein is actually required and essential for the development and progression of TSE disease (Weissmann C. et al., 2003) and genetic variations in the prion gene have been associated with TSE susceptibility in humans (Prusiner S.B., 1998), (Gambetti P. et al., 2003), sheep (Laegreid W.W. et al., 2008) and deer (Blanchong J.A. et al., 2009), (Wilson G.A. et al., 2009). Although amino acid differences in the prion protein are a major contributor to susceptibility and/or resistance risk factor in humans (Spudich S. et al., 1995) and sheep (Baylis M. et al., 2004), this is not the case for cattle. Bovine codon E211K, analogous to codon E200K in human CJD, has only been observed twice in cattle, the first of these cases is associated to atypical BSE and the other case is the offspring of the first (Heaton M.P. et al., 2004; Nicholson E.M. et al., 2008).

The bovine prion gene contains more than 390 SNPs in the 25-kb region of chromosome 13 containing the *PRNP* gene. This chromosomal segment contains distinct regions of high and low LD (Linkage Disequilibrium) that is conserved across many *B. taurus* cattle populations (Clawson M.L. et al., 2006).

The region of high LD includes the promoter region, exons 1 and 2, and part of intron 2 (6.7 kb) of the *PRNP* gene. A 23-bp insertion/deletion (indel) polymorphism in the promoter contains a binding site for the repressor protein 58 (RP58) and a 12-bp indel in intron 1 has a binding site or the transcription factor specificity protein 1 (SP1). The presence or absence of these binding sites modulate the expression of *PRNP* and possibly the expression of PrP in species. Expression of the cellular PrP is necessary for the transmission and propagation of prion diseases (Montrasio F. et al., 2000). Based on reporter gene assays, increased level of PrP decreases the incubation period for cBSE (Sander P. et al., 2005). Susceptibility or resistance to TSE, is associated with variations in

the non-coding region of *PRNP*, including the promoter and/or enhancer. Nucleotide changes in the non-coding region may affect mammalian PrPC expression, which is also known to affect TSE susceptibility or resistance (Sander P. et al., 2004). These polymorphisms are a 23-bp indel at position -1594 and a 12-bp indel at position +300 (the positions of the polymorphisms are given with respect to the transcription start site in GenBank accession No. AJ298878); both are associated with promoter activity and bovine PrPC expression levels in vitro (Sander P. et al., 2005). Therefore, these polymorphisms can both seemingly influence BSE incubation times and susceptibility. So, both the 23- and 12-bp indels that have been associated with C-BSE susceptibility are contained in this region of high LD (Sander P. et al., 2004; Vernerova K. et al., 2014). Several studies have shown the impact on BSE sensitivity in cattle of a 23-bp insertion-deletion (indel) polymorphism located 1.6 kbp upstream of exon 1, and a 12-bp indel inside intron 1 (Haase B. et al., 2007). Although it is clear that cattle have both been substantially correlated with BSE with the -/-23 bp promoter genotype and the -/- 12 bp Intron 1 genotype, there is no agreement about which genotype is most closely linked to BSE (Sander P. et al., 2004). In addition, indel polymorphisms affecting the sensitivity of classical BSE tend not to be relevant in cattle to other transmissible spongiform encephalopathies (Brunelle B.W. et al., 2007). To date, in some cattle in Asia (Nakamitsu S. et al., 2006), Europe (Juling K. et al., 2006) and United States of America (Seabury C.M. et al., 2004), the frequency of polymorphism in the *PRNP* gene promoter region has been identified. Due to their poor milk and meat output, the number of local cattle breeds in Turkey has been declining.

On the other hand, on various platforms, their high tolerance to diseases and parasites is rated (Bakır G. et al., 2003). For example, according to Imran et al [74], Pakistani cattle are relatively more resistant to classical BSE than European cattle. However, the key risk factor for classical BSE is the dietary exposure of susceptible cattle to contaminated feedstuffs.

The remainder of *PRNP*, including the entire coding region, has relatively low LD. To account for the genetic architecture of the *PRNP* gene, a set of haplotype-tagging single-nucleotide polymorphisms (htSNPs) has been described that efficiently define haplotypes within and across each of the LD regions (Clawson M.L. et al., 2006). These SNPs were used to test for association between *PRNP* haplotypes and

susceptibility to either C-BSE or atypical BSE susceptibility (Murdoch B.M. et al., 2010). Susceptibility or resistance to a TSE disease can be influenced by at least 3 factors related to the host prion protein: protein expression levels, the number of octapeptide repeats, and specific amino acid differences. These 3 factors are all relevant to prion biology in cattle. Non-coding region polymorphisms in cattle have been identified that modulate expression level and influence susceptibility to cBSE (Sander P. et al., 2004; Juling K. et al., 2006) but not atypical BSE (Brunelle B.W et al., 2007).

The presence of additional octapeptide repeats in transgenic mice (Castilla J. et al., 2005) and Brown Swiss cattle (Sauter-Louis C. et al., 2006) (have been reported to result in increased susceptibility to classical BSE. Amino acid differences are a major component in susceptibility and resistance to acquired TSE disease in sheep and are the basis for genetic TSEs in humans (Mead S., 2006).

However, studies in cattle revealed that regions outside of the open reading frame are associated with variation in disease susceptibility. While a few studies identified the octapeptide-repeat region, (Sander P. et al., 2005) genetic analyses primarily identified two insertion/deletions in promoter regions of the prion gene, (Vernerova K. et al., 2014) associated with BSE susceptibility and/or resistance. For example a 23-bp insertion/deletion (indel) polymorphism in the promoter contains a binding site for the repressor protein 58 (RP58) and a 12-bp indel in intron 1 has a binding site for the transcription factor specificity protein 1 (SP1). A study conducted by (Murdoch B.M. et al., 2015) examined these SNPs and the 12-bp and 23-bp indels to test *PRNP* haplotypes for an association with C-BSE in 330 European Holstein cows from the U.K. BSE epidemic, of which 146 were BSE cases and 184 were controls. A combination of sequencing, SNP assay (Illumina goldengate assay), and polymerase chain reaction amplification was used to genotype 18 SNPs and 2 indels in 95 BSE case and 134 control animals (Murdoch B.M. et al., 2010).

The presence or absence of these binding sites modulate the expression of *PRNP* and possibly the expression of PrP in species. Expression of the cellular PrP is necessary for the transmission and propagation of prion diseases (Montrasio F. et al., 2000). Based on reporter gene assays, increased level of PrP decreases the incubation period for cBSE (Sander P. et al., 2005). Furthermore, that two bovine *PRNP* alleles have been associated with susceptibility to C-BSE (classic BSE): a 23-

bp deletion within the promoter region and a 12-bp deletion within intron 1 (Sander P. et al., 2004; Juling K. et al., 2006; Hills D. et al., 2001; Vernerova K. et al., 2014).

However, the deletion alleles are not entirely independent of one another as there is high linkage disequilibrium (LD) between the two polymorphic sites in *Bos taurus* cattle populations. This suggests that the possible effects of variations in the *PRNP* gene on incidence of C-BSE may be better understood if *PRNP* haplotypes were considered in testing for association with disease incidence. Moreover, *PRNP* haplotypes, containing one or both of the two insertion/deletion alleles, may have a stronger association with either susceptibility or resistance to C-BSE than if the insertions and deletions (indels) are considered independently.

CONCLUSIONS

Modifications in the prion protein cause prion diseases such as scrapie in sheep, BSE in cattle, and CJD in humans. As is the case with prion diseases, pathogens that infect multiple species can leap species boundaries and impact endangered species. It is known that there is no preventive therapy in other species for BSE in cattle or for prion diseases. Therefore, in the cattle population, genetic selection is a special tool for eradicating BSE. The only sustainable solution is using in reproduction only those individuals that are resistant to BSE. This could be a good strategy for eradicating BSE.

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