Chapter 8

Genetic Resistance to Prion Diseases

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Additional information is available at the end of the chapter

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Abstract

Prions are abnormal isoforms of the host-encoded cellular prion proteins which are misfolding in its three-dimensional structure acquire pathogenicity. Prions cause transmissible spongiform encephalopathy (TSEs) in humans and some animal species including sheep, goats, cattle, cat, deer and elk. TSEs, also called "prion diseases," cause irreversible neurodegeneration in the central nervous system and are always fatal. Cellular prion proteins are encoded by prion protein gene (*PRNP*) in mammals; moreover, it is known that the variations in the *PRNP* gene have influence on the resistance and/or incubation period of the TSEs. It is well-documented that after exposure to the pathogenic prions, development of some TSEs depend on the host *PRNP* genotype, for example, scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt-Jakob disease (CJD) and kuru in humans, as well. In this chapter, genetic resistance to prion diseases will be reviewed.

Keywords: TSE, prion disease, PRNP, genetic resistance

1. Introduction

It is known that conformational changes in prion protein cause Creutzfeldt-Jakob disease (CJD) in humans, scrapie disease in sheep and goats [1, 2], bovine spongiform encephalopathy (BSE) in cattle, feline spongiform encephalopathy in cat, and wasting disease in deer and elk.

Polymorphisms inside the prion protein-coding gene (*PRNP*) in humans and also in some mammalian species have been appeared to impact disease susceptibility and pathologies [3]. In human population, kuru and CJD are profoundly related with polymorphism in codon 129. All CDJ affected individuals are known to be homozygous for methionine amino acid in codon 129 while at the same codon heterozygote individuals seem most resistant to kuru [4, 5]. Also, it is known that there is a high correlation between the polymorphisms in codons

136, 154, and 171 of the PRNP gene and the level of susceptibility to scrapie in sheep [3, 6, 7]. In cattle, numerous studies were carried out for discovering a relationship amongst BSE and polymorphisms in cattle genome [8–12]. The studies about BSE-affected animals in Germany and USA represented the influence of *PRNP* promoter polymorphisms on BSE susceptibility in cattle [13, 14]. The impacts of insertion-deletion (indel) polymorphisms within a location 1.6 kbp upstream of exon 1 and inside intron 1 (23-bp and 12-bp, respectively) on BSE susceptibility are determined by further analyses in cattle [15–17]. Despite the fact that cattle with the -/-23 bp promoter genotype and the –/12 bp intron 1 genotype have both been significantly connected with BSE, it could not be reached any consensus on which genotype is most identified with BSE [13, 15, 16, 18]. In addition, indel polymorphisms that affect the sensitivity of classical BSE appear not to be pertinent to other transmissible spongiform encephalopathies in cattle [19]. Until now, the incidence of *PRNP* gene promoter polymorphisms has been identified in some cattle in Asia [20, 21], Europe [13, 16, 18, 22] and America [14, 23].

2. Resistance in humans

There exist various types of human prion disease such as Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), and Gerstmann Sträussler-Scheinker syndrome (GSS). Related to the cause of the illness they exist in three main forms: Genetic, sporadic and acquired. Genetic form of the disease is caused by a mutation in prion protein-coding gene (*PRNP*), whereas acquired form occurs by the transmission of disease from an animal or another human disease. The cause of sporadic form is not clear up to now [24–26].

The human prion-coding gene consists of two exons and the second one contains the whole open reading frame. It is known that a valine amino acid at position 129 of the human prion protein provide resistancy to the Creutzfeldt-Jakob disease. Both Valin129Valin and Methionine129Methionine genotypes are resistant to the disease, whereas Methionine129Methionine genotypes are susceptible [27, 28]. Another polymorphism at codon 219 was reported to be related with development of Creutzfeldt-Jakob disease in Japanese population [29].

3. Resistance in small ruminants

Scrapie is a neurodegenerative disease of sheep and goats. As with other transmissible spongiform encephalopathies (TSE) which affect humans and animal species, scrapie is always fatal and characterized by long incubation periods ranging from months to years, vacuolation, neuronal loss and astrocytosis in the central nervous system (CNS) and has no inflammatory or immune responses [30]. The earliest reports of the scrapie based on middle of 1700s in Britain. Various terms such as “scrapie,” “scratchie,” “rubbers,” “rickets” and “goggles” were used to indicate the disease [31].

It is thought that scrapie first occurred in the United Kingdom in the eighteenth century and following decades, particularly after World War II, the disease spread by importation of the

infected animals. Scrapie has reported nearly all over the world, for example, Iceland (1878), Canada (1938), USA (1947), Australia (1952), Norway (1958), India (1961), Republic of South Africa (1966), Kenya (1970), Germany (1973), Brazil (1978), Yemen (1979), Sweden (1988), Cyprus (1989) and Japan (1990), reviewed in reference [30].

Scrapie has been known for over 250 years; therefore, it is regarded to be prototype of the TSEs [30]. Earlier, researchers thought that it was a hereditary disease, but later, according to the results of the experimental transmission studies, they were considered that "Scrapie was a natural infection and gained from ground". After seven years of working with several thousand breeding ewes within several hundred ewes were affected classical scrapie, H. B. Parry postulated some hypothesis that scrapie had a hereditary feature in a simple Mendelian autosomal recessive manner, development of the disease determined by genotype of the individuals, and it was not a natural infection. They observed that in high-incidence flocks, many scrapie diseased individuals had affected parent or progeny [32, 33]. Later studies revealed the evidences that scrapie is a transmissible infection [34] which is caused by a kind of proteins called "prion" [35], and development and/or incubation period of the disease under genetic control [36–40].

3.1. Resistance in sheep

Sheep and goat prion protein-coding gene (*PRNP*) which encodes the cellular prion protein located on chromosome 13 [41]. The gene structure of the sheep *PRNP* was determined by [40], they demonstrated that sheep *PRNP* encoded 256 amino acids and highly homologous with the *PRNP* gene of the other species. Furthermore, the authors suggest that arginine/glutamine substitution in the 171th position of the sheep *PRNP* might have affected the scrapie incubation period. According to the results of many subsequent study polymorphisms of 136th, 154th and 171th codons of ovine *PRNP* had a strong influence on susceptibility or resistance to the scrapie [8, 42–45].

Commonly encoded amino acids at three codons are as follows: alanine (A) or valine (V) at codon 136, arginine (R) or histidine (H) at codon 154 and glutamine (G), histidine (H) or arginine (R) at codon 171 and out of possible other combinations, common *PRNP* alleles are A136R154R171, A136R154Q171, A136R154H171, A136H154Q171 and V136R154Q171, (respectively, ARR, ARQ, ARH, AHQ and VRQ for short) [45, 46]. While ARR alleles related to resistance, VRQ is regarded as the most susceptible alleles. Until now, only three scrapie cases were reported in ARR homozygous sheep which are one case from Japan [47] and two cases from France and Germany [48]. Some studies on PrP genotype and their relevance to scrapie in scrapie diseased sheep are presented in **Table 1**.

There is no report about direct transmission from sheep to human in natural condition, nevertheless, scrapie can be transmitted interspecies by experimentally [59–61], furthermore, the cattle prion disease, Bovine spongiform encephalopathy (BSE) which is transmitted to human and causes a variant of Creutzfeldt-Jakob disease (vCJD) [62], originated from the usage of scrapie contaminated material in cattle nutrition [63]. Even, in a more recent study, natural scrapie isolate was successfully transmitted to a primate (cynomolgus macaque) suggesting that scrapie has zoonotic potential to primates including human [64]. Epidemiological connection with scra-

Risk groups	PrP Genotypes	Norway *n* = 32 [49]	England *n* = 21 [50]	England *n* = 59 [51]	France *n* = 437 [52]	France *n* = 245 [53]	Ireland *n* = 154 [54]	Italy *n* = 34 [55]	The Netherlands *n* = 34 [45]	Iceland *n* = 101 [56]	Greece *n* = 216 [57]	Japan *n* = 15 [47]	Canada *n* = 249 [58]
1	ARR/ARR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.000
2	ARR/AHQ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
	ARR/ARH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000
	ARR/ARQ	0.063	0.000	0.000	0.005	0.008	0.000	0.000	0.000	0.000	0.120	0.066	0.000
3	ARQ/ARH	0.000	0.000	0.000	0.000	0.041	0.162	0.000	0.000	0.000	0.000	0.000	0.000
	ARQ/AHQ	0.000	0.000	0.017	0.016	0.004	0.000	0.059	0.000	0.000	0.176	0.000	0.004
	AHQ/AHQ	0.063	0.000	0.017	0.002	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	ARH/ARH	0.000	0.000	0.000	0.000	0.004	0.006	0.000	0.000	0.000	0.005	0.000	0.000
	AHQ/ARH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	ARQ/ARQ	0.031	0.143	0.136	0.210	0.371	0.422	0.941	0.088	0.465	0.509	0.867	0.916
4	ARR/VRQ	0.000	0.095	0.254	0.020	0.070	0.006	0.000	0.029	0.000	0.000	0.000	0.012
5	AHQ/VRQ	0.000	0.000	0.000	0.007	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.004
	ARH/VRQ	0.000	0.286	0.051	0.000	0.037	0.026	0.000	0.441	0.000	0.000	0.000	0.000
	ARQ/VRQ	0.156	0.476	0.407	0.470	0.371	0.363	0.000	0.353	0.406	0.000	0.000	0.052
	VRQ/VRQ	0.688	0.000	0.119	0.280	0.086	0.013	0.000	0.088	0.129	0.079	0.000	0.012

Table 1. PrP genotype frequencies of the scrapie-infected sheep in various countries.

pie, BSE and vCJD emerged public health concerns and lead to establishing scrapie eradication programs, including increasing the genetic resistance to scrapie in scrapie epidemic countries.

In 2001, Great Britain has established the "National Scrapie Plan" (NSP) intending to increase the frequencies of resistance alleles by selective breeding and eventually eradicate scrapie from British sheep herds. According to disease-associated alleles, five risk groups were designated from R1 to R5 where is R1 referring at the lowest risk and R5 at highest risk [65]. NSP scrapie risk groups can be seen in **Table 2**.

Reported case per year and estimated of the case number per million sheep according to risk groups in the United Kingdom (UK) are given in **Table 3**.

European Union (EU) Commission has issued a regulation in 2003 that required the establish of a selective breeding program for resistance to TSE in each sheep breed of member states [66]; therefore, European member states have been implementing breeding programs based on elimination of the most susceptible alleles while increasing resistant allele frequencies. For example, as a result of intensive genetic selection programs, particularly in high genetic merit flocks, ARR allele frequencies increased from 50 to 69% in the UK, 49 to 85% in France, 38 to 70% in the Netherlands and 47 to 70% in Italy [67].

Risk groups	Genotype of individuals	Degree of resistance/susceptibility
R1	ARR/ARR	Sheep that are most resistant to scrapie
R2	ARR/AHQ	Sheep that are resistant to scrapie, but will need careful selection when used further breeding
	ARR/ARH	
	ARR/ARQ	
R3	ARQ/ARH	Sheep that have little resistance and will need careful selection when used for further breeding
	ARQ/AHQ	
	AHQ/AHQ	
	ARH/ARH	
	AHQ/ARH	
	ARQ/ARQ	
R4	ARR/VRQ	Sheep that are susceptible to scrapie and should not be used for breeding because of carrying VRQ allele
R5	AHQ/VRQ	Sheep that are highly susceptible to scrapie and should not be used for breeding
	ARH/VRQ	
	ARQ/VRQ	
	VRQ/VRQ	

Table 2. PrP genotypes and allocation of them into scrapie risk groups (adapted from reference [65]).

Risk groups	Case per year (*n*)	Percentage of sheep	Case per year per million (*n*)
R1	0	21.3	0
R2	2.3	35.7	0.7
R3	104.9	23.9	57.8
R4	12	9.6	6.3
R5	381.8	9.6	1175.6

Table 3. Estimates of the number of reported cases of scrapie per million sheep of each risk groups in the UK (adapted from reference [46]).

Given the importance of the disease, a lot of genotyping studies on sheep *PRNP* have carried out in the almost all over the world such as; in New Zealand and Australia [68], Brazil [69], Israel, Palestine, and Jordan [70], Turkey [71], Egypt and Saudi Arabia [72] and East Asia [73], whether scrapie have reported or never been reported.

3.2. Resistance in goats

First natural scrapie case in goats was defined in 1942 [74]. Although goat scrapie has rare incidence compared with sheep, a surveillance program between 2002 and 2009 was performed according to the EU commission direction and over 3000 scrapie cases were reported in goats [75]. Scrapie cases occurring in natural condition in goats have been reported, particularly throughout Europe [76–78]. Transmission of the scrapie from naturally affected sheep to goats which rearing together has often been observed [77, 79–81], in addition, transmission from goat to goat has been known [76].

In contrast to sheep, limited data are available related to scrapie resistance and *PRNP* alleles. Genotyping studies on goats *PRNP* have given various results in terms of disease susceptibility or resistance. Assessment of *PRNP* alleles in scrapie infected and non-infected goats presented in **Table 4**.

As provided in **Table 4**, some relationships between caprine *PRNP* polymorphisms and scrapie resistance were defined. Encoding of serine instead of glycine at codon 127 has decreased the probability of clinical manifestation of the disease [86]. Isoleucine-methionine dimorphism at codon 142 has found to be associated both experimental [88] and natural infection [86, 89]; furthermore, it is reported that [89] the presence of methionine-isoleucine as heterozygous at codon 142 has been provided resistance only in proline-proline homozygous animal at codon 240. Encoding of arginine at codon 143 has provided limited protection to natural scrapie [80]. While the presence of asparagine instead of Serine or Aspartic acid at codon 146 has been found to be related to susceptibility to natural infection [78], it also has reported that the presence of Serine as heterozygous at the same codon has associated with the extended incubation period in oral challenging [90]. According to the results of various studies, arginine-histidine dimorphism at codon 154 has provided limited resistance [78, 80, 83, 89]. The presence of glutamine/arginine as heterozygous at codon 211 has been found to

Codons	AA substitution	Association to disease	References
18	W-R		[82]
21	V-A		[80]
23	L-P		[80]
37	G-V		[83, 84]
49	G-S		[80]
101	Q-R		[82]
110	T-P		[83, 84]
127	G-S	Incubation period/resistance	[85, 86]
133	L-Q		[93]
137	M-I		[93]
139	R-S		[87]
142	I-M	Incubation period	[84, 86, 88, 89]
142	I-T		[84]
143	H-R	Limited resistance	[80, 88]
145	G-D		[87]
146	N-S or D	Resistance	[78, 90]
151	R-H		[78]
154	R-H	Limited resistance	[78, 80, 83, 89]
168	P-Q		[80]
194	T-P		[84]
201	F-L		[86]
208	R-Q		[91]
211	R-G		[85]
211	R-Q	Lower susceptibility	[84, 89]
219	T-I		[92]
220	Q-H		[80]
222	Q-K	Resistance	[83, 89, 90, 93]
232	G-W		[82]
240	S-P	Resistance (connected with codon 142)	[88, 89]

Abbreviations of the amino acids: A, alanine; D, aspartic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan.

Table 4. The *PRNP* polymorphisms of scrapie-infected/noninfected goats and association of polymorphisms with scrapie resistance.

be related to lower susceptibility [89], and the presence of lysine at codon 222 has been associated with resistance to both natural [83, 89, 93] and oral [90] or intracerebral challenging [94].

Apart from these polymorphisms, an allele of caprine *PRNP*, which encodes shorter cellular prion protein, has been reported. An experimental transmission to a goat carrying this allele as heterozygote has died after an unusually long incubation period [95]. In addition, a novel 28 bp insertion in the promoter region of caprine *PRNP* was found by [96] in healthy Chinese native goat breeds. Although there is no information with respect to disease resistance, some associations between this insertion/deletion polymorphism and production trait were reported.

Influences of the remaining codons over scrapie resistance or susceptibility in goats are not known yet. Currently available data on genetic resistance to scrapie are considered insufficient to establish selective breeding programs in goats.

3.3. Atypical scrapie in sheep and goats

Norwegian researchers have recognized a novel type of scrapie case in 1998 which has unusual histopathological features comparing with classical scrapie. The geographical distribution of the disease indicated that it might be spontaneous scrapie, not a contagious disease. This atypical form of scrapie designated as Nor98 by the authors [97]. Later studies conducted on archived tissue specimens revealed that atypical scrapie is not a new disease and has been existed at least from late 1980s in the UK herds [98, 99]. In the following years, many atypical scrapie cases were reported in sheep and/or goats from [100–103], North America [104] and New Zealand [105], as well.

Atypical cases have appeared to relate with the *PRNP* genotypes considered relatively resistant to classical scrapie. Sheep which are carrier of AHQ allele have found to be more susceptible to atypical scrapie; moreover, unlike classical scrapie, it was demonstrated that the presence of phenylalanine at codon 141 strongly associated with atypical cases [51, 53, 100, 106–109]. Interestingly, according to results of case control studies, while VRQ allele which is the most classical scrapie have found to be related to low incidence in atypical scrapie [51, 53, 108], the most resistant ARR allele associated with higher incidence [53, 107, 109]. Distribution of *PRNP* genotypes and roles of codon 141 on atypical scrapie resistance demonstrated in **Table 5**.

Although there is very limited data about relationship atypical scrapie and *PRNP* genotypes in goats, it has been reported that the presence of histidine at codon 154 may associated with atypical cases in goats, as well [103, 109].

European selective breeding programs against to classical scrapie in sheep already eliminating the AHQ and AFRQ alleles which have demonstrated to relate with atypical scrapie susceptibility; however, the major problem about ARR (resistant to classical scrapie but susceptible to atypical scrapie) and VRQ (susceptible to classical scrapie but resistant to atypical scrapie) alleles remains to be solved.

Risk groups for classical scrapie	Genotype of individuals	*n* = 38 [106]	*n* = 69 [51]	*n* = 51 [109]	*n* = 248 [53]
R1	ARR/ARR		0.129	0.118	0.181
R2	ARR/AHQ	0.132	0.217	0.039	0.097
	ARR/ARH		0.014		0.012
	ARR/ARQ		0.029	0.039	0.040
	ARR/**AFRQ**	0.105	0.101	0.314	0.218
R3	ARQ/ARH				
	AFRQ/ARH				0.004
	ARQ/AHQ	0.053	0.174	0.020	0.052
	AFRQ/AHQ	0.211	0.072		0.044
	AHQ/AHQ	0.211	0.145	0.039	0.024
	ARH/ARH			0.020	0.004
	AHQ/ARH	0.026		0.020	0.008
	ARQ/ARQ	0.053			0.008
	ARQ/**AFRQ**	0.079	0.014	0.176	0.173
	AFRQ/AFRQ	0.132	0.087	0.137	0.113
R4	ARR/VRQ				
R5	AHQ/VRQ			0.020	0.004
	ARH/VRQ				0.004
	ARQ/VRQ				
	AFRQ/VRQ		0.014	0.059	0.012
	VRQ/VRQ				

Table 5. *PRNP* genotypes according to codons 136, 154 and 171 (and codon 141 if the presence of phenylalanine residue) and association with atypical scrapie.

4. Resistance in cattle

Bovine spongiform encephalopathy (BSE), the cattle prion disease, belongs to animal TSE's which has been characterized histopathological changes in the CNS as with scrapie. It is newly diagnosed prion disease, which has been never known until 1986 [110]. BSE became epidemic during the 1980s in the UK as a result of the changing rendering process and allowing to enter the prion contaminated product to cattle nutrition, and it is estimated that the exposure began in the early 1980s [110]. Having transmitted to human and causing a new variant of Creutzfeldt-Jakob disease (CJD) [62] which is a human prion disease acquired from consumption of the meat products of the BSE diseased cattle [111], BSE has been regarded by the World Health Organization [112] as zoonotic. Unlike CJD, vCJD has diagnosed in younger

people in the UK [113], latter in France [114]. Up to 2003, 135 vCJD cases have reported from the UK and 6 cases from France (reviewed in reference [115]).

BSE could transmit to sheep and goats by experimental routes [116] and development of the disease seemed to be affected by the *PRNP* genotype of the individual [88, 117]; furthermore, it was reported that BSE in goats can be occur in natural conditions [118, 119].

Because of the zoonotic potential and the ability to spread between species of the BSE, it has raised the public health concerns and enforced to governments to take control and preventive measures; moreover, researchers have intensified to reveal the genetic background of the disease.

Early studies on association between *PRNP* genotype of cattle and development of the BSE have focused on two known polymorphisms; the HindII restriction site and an octapeptide repeated sequence in the coding region of the cattle *PRNP*, but no relationship between these genotypes and BSE infection has found [120, 121]; however, although lack of detailed genetic information, some clues were obtained suggesting that BSE might be in linkage with host *PRNP* genotype [9].

In the following years, hundreds of nucleotide changes and insertions/deletions (indel) were identified in bovine *PRNP* [13, 122, 123, 124], including a 12 base pair (bp) indel within the intron 1 and a 23 bp indel within the promoter region [13, 122]. Case control studies showed that distribution of these two indel polymorphisms were different between healthy and BSE affected cattle and insertion alleles presumably connected with disease resistance [13]; moreover, it has demonstrated that insertion alleles related to the lower prion protein level compared with deletion alleles and may differentiate of the BSE incubation period [15]. Further studies have supported the relationship between BSE resistance and 23 bp/12 bp indel genotypes that are given in **Table 6**.

Although the clear association has been shown between *PRNP* indel genotypes and BSE incidence, there are some paradoxical situations at breed level, for example, it was reported that although Brown breeds have higher allelic frequency of insertion alleles, at the same time, these breeds have higher prevalence of BSE [17]. However, beside of the primary measures for prevention from circulation of BSE agents and exposure to both animal and human, selective breeding can offer a secondary strategy to eliminate the BSE.

Apart from classical BSE, two more types of the disease have been diagnosed by histopathological examinations; H-type and L-type, both of two types classified as atypical BSE and have been observing sporadically. While H-type BSE characterized with higher molecular mass [126], L-type BSE which is also named as bovine amyloidotic spongiform encephalopathy (BASE), characterized with lower molecular mass and has diverse glycopattern of pathogenic prion proteins [127].

It is reported that *PRNP* 23 and 12 bp indel polymorphism do not provide the genetic resistance, neither to naturally occurring atypical BSE nor to experimentally inoculated other TSEs [16]. Although very limited data, several atypical cases with extremely rare [128] glutamate to lysine mutation in codon 211 (E211K), which is homologous with human E200K mutation in

	23 bp indel genotypes								
	Healthy cattle				BSE-affected cattle				
Breed	*n*	**in/in**	**in/del**	**del/del**	*n*	**in/in**	**in/del**	**del/del**	**References**
Pooled German breeds	48	0.210	0.440	0.350	43	0.050	0.440	0.510	[13]
UK Holstein	276	0.047	0.489	0.464	363	0.013	0.410	0.554	[16]
German Holstein	313	0.147	0.473	0.380	127	0.079	0.465	0.457	[16]
German Brown	87	0.448	0.414	0.138	43	0.140	0.651	0.209	[16]
German Fleckvieh	136	0.103	0.434	0.463	106	0.066	0.396	0.538	[16]
Pooled German and Switzerland breeds	574	0.160	0.470	0.370	670	0.090	0.470	0.450	[17]
Pooled Japanese breeds	464	0.071	0.440	0.489	6	0.000	0.333	0.467	[20]
Pooled Czech breeds	81	0.235	0.543	0.222	26	0.077	0.538	0.385	[125]
	12 bp indel genotypes								
	Healthy cattle				**BSE-affected cattle**				
Breed	*n*	**in/in**	**in/del**	**del/del**	*n*	**in/in**	**in/del**	**del/del**	**References**
Pooled German breeds	48	0.210	0.560	0.230	43	0.090	0.470	0.440	[13]
UK Holstein	270	0.111	0.519	0.370	350	0.051	0.454	0.494	[16]
German Holstein	309	0.220	0.498	0.282	125	0.144	0.456	0.400	[16]
German Brown	90	0.744	0.222	0.033	43	0.419	0.512	0.070	[16]
German Fleckvieh	137	0.153	0.453	0.394	106	0.085	0.462	0.453	[16]
Pooled German and Switzerland breeds	574	0.230	0.460	0.310	670	0.170	0.490	0.340	[17]
Pooled Japanese breeds	476	0.095	0.468	0.437	6	0.000	0.333	0.467	[20]
Pooled Czech breeds	81	0.358	0.444	0.198	26	0.231	0.462	0.308	[125]

Table 6. The distribution of the *PRNP* 23 bp indel and 12 bp indel genotypes according to breeds, in both healthy and BSE-affected cattle.

the *PRNP* gene, has determined, suggesting that association to atypical BSE resistance may be exist [129, 130], but could not confirm by following studies [131, 132]. Transmissibility of the H-type atypical BSE to cattle which is carrying the E211K mutation was demonstrated [133], on the other hand, some evidences have obtained that the E211K is a germ line mutation, thus, may cause inherited BSE that can be transmitted genetically [130].

5. Resistance in water buffaloes

During the BSE epidemic in 1980s, it can be assumed that BSE and/or scrapie contaminated by-products most likely have entered in to water buffalo (Bubalus bubalis) nutrition systems, as well. EU member states have approximately 409 thousand of buffaloes, where 90% of those have been reared in Italy [134]. Between 2001 and 2005, 128 BSE cases in cattle have been reported from Italy [135]. Along with cattle, bison, sheep, goats and some exotic ruminants, water buffaloes have been considered as TSE-related risk factors [136]; nevertheless, no BSE or any other TSE has ever been reported in water buffaloes [137] neither in Italy nor the rest of the world.

Only few studies on indel polymorphisms of the water buffalo *PRNP* gene have conducted to compare with cattle *PRNP*. According to the results, 12 and 23 bp indel polymorphisms have been existed in water buffalo, as well. Furthermore, insertion alleles which are relate to BSE resistance have observed more frequent than those in cattle [138–141] that is given in **Table 7**.

As seen in **Table 7**, almost all buffalo breeds, except Thai river buffalo, are carrying mostly insertion alleles either at 23 or 12 bp indel loci. This may be an explanation for why buffaloes putatively resistant to BSE.

			23 bp indel alleles		**12 bp indel alleles**		**References**
Country	**Breed**	*n*	**In %**	**Del %**	**In %**	**Del %**	
Turkey	Anatolian Buffalo	106	92	8	86	14	[138]
Pakistan	Nili Buffalo	66	94	6	86	14	[139]
	Ravi Buffalo	39	97	3	83	17	
	Azikheli Buffalo	20	100	0	95	5	
	Kundhi Buffalo	34	97	3	88	12	
	Nili Ravi Buffalo	122	94	6	87	13	
Indonesia	River Buffalo	14	100	0	100	0	[142]
Thai	River Buffalo	45	53	47	84	16	
Germany	River Buffalo	11	100	0	100	0	[140]
Poland	River Buffalo	29	100	0	100	0	
Turkey	Anatolian Buffalo	89	100	0	100	0	[141]
	Murrah Buffalo	20	100	0	100	0	

Table 7. 23 and 12 bp allele frequencies of healthy water buffaloes reared in Asian and European states.

The SPRN gene, which belongs to the prion protein gene family, encodes the shadow protein. Shadow protein shares characteristic features with cellular prion protein, suggesting the existence of a functional relation with prion proteins [143]. A comparative study revealed that the SPRN gene has species-specific indel polymorphisms in cattle and buffaloes and causes different promoter activity and expression levels [144]. Furthermore, according to the results of more recent study, molecular structure of buffalo cellular prion protein is different from cattle, but similar to those of rabbits, dog and horse which are considered low susceptible to TSEs [145]. These molecular and structural differences may be another explanation with regard to TSEs resistance in buffaloes.

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