Survey of Ovine Prion Protein (PRNP) Polymorphisms in Oklahoma Sheep

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Story in Brief

One thousand one hundred forty-four sheep belonging to 21 breeds and known crosses from flocks across Oklahoma were genotyped for polymorphisms in ovine prion protein (PRNP) gene. Certain polymorphisms in PRNP are known to confer resistance to scrapie, a fatal neurodegenerative disease in sheep. We identified polymorphisms at codons 136(A/V), 154(H/R), and 171(Q/R/H/K) that play a role in resistance to scrapie. The frequency of 171R allele known to confer resistance to type C scrapie was 53.8% and the frequency of the 136A allele known to influence resistance to type A scrapie was 96.01%. At the present time 78.3% of sheep in Oklahoma carry resistant genotypes (RR and QR) at codon 171 for type C scrapie that is prevalent in the US. In addition we report the identification of five new polymorphisms at codons 143(H/R), 167(R/S), 180(H/Y), 195(T/S) and 196(T/S). We also report the identification of a novel allele (S/R) at codon 138.

Key Words: sheep, scrapie, prion, polymorphisms, resistance, genotyping,

Introduction

Scrapie, a fatal neurodegenerative disease in sheep and goats, is a member of the mammalian transmissible spongiform encephalopathy (TSE) disease family. Other members of this disease family include, Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy (TME), chronic wasting disease (CWD) in deer and elk and feline spongiform encephalopathy (FSE) (Prusiner, 1998). The causative agent for scrapie is believed to be a protease-resistant isoform of sheep prion protein (PrP^{sc}), which is derived from an endogenous, protease-sensitive precursor (PrP^{c}) (Prusiner, 1982, 1996). Polymorphisms within the prion gene (PRNP) are associated with susceptibility/resistance to TSEs in sheep (Hunter et al., 1997), goats (Goldmann et al., 1996) and in humans (Mead et al., 2003). There are over 15 polymorphisms reported in PRNP (DeSilva et al., 2003). Of these, only three codons (codon 136, 154 and 171) have been reported to affect the susceptibility to the disease. Susceptibility to ovine scrapie is also determined by the infective scrapie strain (O'Rourke et al., 1997). Two strains of scrapie have been defined. Type A produces the disease in sheep that are either homozygous or heterozygous for a valine at codon 136 while type C causes disease in sheep that are homozygous for a glutamine at codon 171. Type C scrapie is considered to be the prevalent form in the United States.

In the United States, the Suffolk breed accounts for 86.4% of all scrapie cases. From the time it was first diagnosed in 1947 to 2001, approximately 1,600 sheep and seven goats have been diagnosed with scrapie in the US and the annual loss to the US sheep industry due to scrapie is estimated to be US$20 million per year (Kahler, 2002)

There has been an aggressive effort to eradicate scrapie from US sheep populations by eliminating the scrapie susceptible genotypes from flocks by selecting for the R allele at codon

171. The objective of this study was to identify prion protein polymorphisms among sheep in Oklahoma and to ascertain the frequency of alleles that confer resistance to the disease. This is the largest survey of PRNP polymorphisms reported in the US.

Materials and Methods

Animals and Sample Collection. One thousand one hundred forty-one sheep from flocks across Oklahoma were included in this study. Owners of these animals participated in a voluntary scrapie certification program and they were instructed to collect a few drops of blood in an Isocode Stix DNA isolation matrix (Schleicher and Schuell, Dassel, Germany), dry at room temperature, and mail to the laboratory. All samples were collected during 2002-2003. Oldest animal reported was 6 years old and there are several new born lambs. Male to female ratio was 1:15.5. These animals are representative of the average breed distribution of meat breeds of sheep in Oklahoma. Suffolk with 232 animals was the most numerous breed represented followed by Hampshire (n=106), Dorset (n=88) and Montadale (n=54). There were a total of 21 breeds and known crosses with most representing less than 10 animals per breed. They were pooled as meat type crosses, wool type crosses and hair breeds for ease of analysis. Meat type crosses included Oxford, Suffolk, Dorset, Montadale and Hampshire crosses. Wool type crosses were Romney, Merino, Rambouillet and their crosses. Hair types were St. Croix, Dorper, Katahdin, Barbados, and their crosses.

DNA extraction and PCR amplification and sequencing. DNA was isolated from the matrix as previously described (DeSilva et al., 2003) and a 421-bp fragment from the PRNP gene was PCR amplified. Amplified PCR products in 96-well format were treated with shrimp alkaline phosphatase and exonuclease III (Amersham Biosciences, Piscataway, NJ) to eliminate excess primers and nucleotides. PCR products were subsequently sequence analyzed on an ABI 3700 DNA analyzer using a nested primer.

Data analysis. All sequences were base-called and analyzed using the Phred/Phrap/Consed suite of programs. PolyPhred (Ver 4) was used to identify single nucleotide polymorphisms (SNPs) in the assembled sequences (DeSilva et al., 2003). All data were manually checked for accuracy at codons where polymorphisms were detected. Any ambiguous codon identifications were discarded from the final analysis.

Results and Discussion

PRNP allele variants for 1144 sheep are reported here. We observed four alleles and six different genotypes at codon 171 (Table 1). Q, R, and H alleles are well known. We identified a novel lysine (K) polymorphism at codon 171 in eight animals (Guo et al., 2003). QQ, QR and RR genotype frequencies were 20.89, 49.13 and 29.20% respectively. According to this data 79.11% of Oklahoma sheep are resistant to type C scrapie. Of the studied major breeds, the R allele frequency was highest in Suffolk (59.05%) and lowest in Dorset (31.25%). Out of the 2288 alleles analyzed, we only found one histidine (H) allele in a St. Croix – Dorper ewe. The K-171 allele was seen in eight animals with seven having KQ genotype and one having KR. The effect of K-171 allele in resistance to type C scrapie remains unknown.

Table 1. PRNP genotypes and allele frequencies at codon 171 in Oklahoma sheep

Breed	Codon 171 Genotype frequency													Allele frequency			
	Total	QQ	%	QR	%	RR	%	KQ	%	KR	%	HQ	%	Q%	R%	K%	H%
Suffolk	232	44	18.97	102	43.97	86	37.07		0.00		0.00		0.00	40.95	59.05	0.00	0.00
Hampshire	106	27	25.47	56	52.83	23	21.70		0.00		0.00		0.00	51.89	48.11	0.00	0.00
Dorset	88	40	45.45	41	46.59	7	7.95		0.00		0.00		0.00	68.75	31.25	0.00	0.00
Montadale	54	22	40.74	24	44.44	8	14.81		0.00		0.00		0.00	62.96	37.04	0.00	0.00
*Meat type crosses	170	32	18.82	86	50.59	49	28.82	3	1.76		0.00		0.00	45.00	54.12	0.88	0.00
*Wool type crosses	26	4	15.38	12	46.15	10	38.46		0.00		0.00		0.00	38.46	61.54	0.00	0.00
*Hair breeds	77	26	33.77	37	48.05	8	10.39	4	5.19	1	1.30	1	1.30	61.04	35.06	3.25	0.65
Unknown	391	44	11.25	204	52.17	143	36.57		0.00		0.00		0.00	37.34	62.66	0.00	0.00
Total	1144	239	20.89	562	49.13	334	29.20	7	0.61	1	0.09	1	0.09	45.80	53.80	0.35	0.04

* See Materials and Methods for breeds pooled in these categories

R allele frequencies and the desirable QR and RR genotype frequencies observed in this study are much higher than that reported previously for US breeds. This is likely due to aggressive selection for the R-171 allele among Oklahoma sheep breeders over the last five years.

We observed the known polymorphisms of alanine (A) and valine (V) at codon 136. AA, AV, and VV genotype frequencies across all animals tested were 93.86, 4.31 and 1.83% respectively (table 2). Frequency of the alanine allele was 96.01% and that of valine was 3.99%. These data are consistent with the observation that V-136 is rare in U.S. sheep populations. Our data are also consistent with the observation that V-136 is extremely rare in Suffolk sheep. Out of 264 Suffolk alleles tested, we identified two V-136 alleles (0.76%). Surprisingly, V-136 allele frequency was

significantly higher in Montadale sheep (22.34%). However, this observation could be biased as most Montadale sheep in the current study (47) came from one flock.

Table 2. PRNP genotypes and allele frequencies at codon 136 in Oklahoma sheep

Breed	Codon 136 Genotype							Allele frequency	
	Total	AA	%	AV	%	VV	%	A%	V%
Suffolk	132	130	98.48	2	1.52		0.00	99.24	0.76
Hampshire	92	92	100.00		0.00		0.00	100.00	0.00
Dorset	63	55	87.30	6	9.52	2	3.17	92.06	7.94
Montadale	47	28	59.57	17	36.17	2	4.26	77.66	22.34
*Meat type crosses	115	114	99.13	1	0.87		0.00	99.57	0.43
*Wool type crosses	16	16	100.00		0.00		0.00	100.00	0.00
*Hair breeds	67	67	100.00		0.00		0.00	100.00	0.00
Unknown	233	216	92.70	7	3.00	10	4.29	94.21	5.79
Total	765	718	93.86	33	4.31	14	1.83	96.01	3.99

* See Materials and Methods for breeds pooled in these categories

We observed known polymorphisms of arginine (R) and histidine (H) at codon 154 (table 3). RR, RH and HH genotypes across all breeds were 99.03, 0.88 and 0.09% respectively. These data were consistent with other studies.

Table 3. PRNP genotypes and allele frequencies at codon 154 in Oklahoma sheep

Breed	Codon 154							Allele frequency	
	Total	RR	%	RH	%	HH	%	R%	H%
Suffolk	233	232	99.57	1	0.43		0.00	99.79	0.21

Hampshire	105	105	100.00		0.00		0.00	100.00	0.00
Dorset	92	90	97.83	2	2.17		0.00	98.91	1.09
Montadale	54	54	100.00		0.00		0.00	100.00	0.00
*Meat type crosses	168	168	100.00		0.00		0.00	100.00	0.00
*Wool type crosses	21	20	95.24	1	4.76		0.00	97.62	2.38
*Hair breeds	76	75	98.68	1	1.32		0.00	99.34	0.66
Unknown	389	383	98.46	5	1.29	1	0.26	99.10	0.90
	1138	1127	99.03	10	0.88	1	0.09	99.47	0.53

* See Materials and Methods for breeds pooled in these categories

Eight additional polymorphisms were identified in this study (Table 4). Two of these polymorphisms (codons 137 and 138) have been described previously. We identified a novel allele at a third known polymorphic codon (codon 138). Five remaining polymorphisms have not been seen in sheep before. The effect of these polymorphisms n scrapie pathogenesis remains unknown.

Table 4. secondary polymorphisms in PRNP gene identified in Oklahoma sheep

Codon	Substitution	Number of animals		Status
		Homozygotes	Heterozygotes	
137	M→T	1	32	Known
138	S→R		15	New Allele
141	L→F		23	Known
143	H→R	6	48	New Polymorphism
167	R→S	1	14	New Polymorphism
180	H→Y		2	New Polymorphism

195	T→S		4	New Polymorphism
196	T→S		2	New Polymorphism

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