PRELIMINARY REPORT: AN EPIDEMIOLOGICAL REVIEW OF A VALINE-ASSOCIATED SCRAPIE OUTBREAK: QUANTIFICATION OF GENETIC RISK AND THE IMPACT OF LATERAL TRANSMISSION IN THE OUTBREAK

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Introduction

Scrapie in sheep and goats, a fatal neurodegenerative disease, is a member of family of transmissible spongiform encephalopathies (TSEs), which includes bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease in humans. Common to all TSE diseases is the accumulation of the abnormal PrPSc form of the normal prion protein PrPC, predominantly in the brain and nervous tissue (Prusiner, 1998). This infectious protein, PrPSc, is thought to be the causative agent of TSE (Prusiner, 1998).

Susceptibility to scrapie is primarily controlled by polymorphisms in the prion protein gene (PRNP). Ovine PRNP has at least five polymorphic sites, resulting in the substitution of one amino acid for another. Three of these polymorphisms are strongly linked to the occurance of natural and experimental scrapie (Baylis, 2000). These are valine (V) or alanine (A) at codon 136, arginine (R) or histidine (H) at codon 154, and glutamine (Q), arginine (R), or histidine (H) at codon 171. Two field strains of scrapie have been identified in sheep. In valine-associated scrapie, valine (V) at codon 136 is linked to scrapie susceptibility, while alanine (A) is linked to resistance (Hunter et al, 1994, 1996). In valine-independent scrapie, glutamine (Q) and histidine (H) at codon 171 are linked to susceptibility, while arginine (R) is linked to resistance (Review by Baylis, 2004).

Lateral transmission, transmission to sheep after birth, either via the environment or by close contact has been shown to occur (Hoinville, 1996). The placenta has been found to be infective (Pattison et al., 1998) and is the presumed source of environmental contamination (Ryder, 2004). The genotype of the conceptus determines the accumulation of the disease specific PrP (Tuo, et al, 2001; Tuo, 2002; Andreoletti, 2002).

The presence of the infectious prion, along with the inheritance of a susceptible genotype, results in the outbreak of scrapie. The predominant form of scrapie in the United States thus far has been associated with the alanine allele at codon 136 (O'Rourke, 1996). The objectives of this study are to characterize an outbreak of valine associated scrapie in a United States flock, quantify the risk of scrapie infection based on allele frequencies at codon 171 and 136, and provide evidence of lateral transmission.

Materials and Methods

Study Population- The flock was identified as a trace back flock for scrapie April 15, 2002, following diagnosis of scrapie in a sheep born in the flock and sold to a private producer. The first infected animal in the flock was diagnosed September 2002. As part of the national scrapie program, North Dakota State University (NDSU) depopulated a number of individuals and genotyped the entire flock. A total of 1006 blood samples from mixed age animals were submitted for genotyping in 2002. In 2003, using either archived DNA or by redrawing blood samples from animals previously tested at codon 171, genotype results at codon 136 were obtained for 842 of the animals. This left 164 animals with unknown genotype results at codon 136 and codon 154. Of these 164 samples, 53% were QQ, 36% were QR, and 11% were RR.

Scrapie diagnosis- A sample of 190 animals from the 842 genotyped animals had a confirmed scrapie status. As part of the flock depopulation, sheep were sent to be slaughtered. The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) collected tonsil, lymph node, and brain tissue from 164 slaughtered animals to determine scrapie status as part of the slaughter surveillance program. The remaining 26 results were determined by Animal Disease Research Unit (ADRU), ARS, USDA in Pullman, WA and confirmed by USDA APHIS as part of the flock depopulation or flock surveillance. Of these 190 samples, females represented 82.6% and males 17.4%. Therefore, convenience samples used were biased toward scrapie positive cases.

Techniques of scrapie status determination- Obex, tonsil, and lymph node tissue were tested using the immunohistochemistry (IHC) at the National Veterinary Service Laboratory (NVSL) and its approved laboratories. A positive case was defined as having a positive test result in any tissue.

Results

Census Results- Of the 842 sheep genotyped, 172 (20.4%) were at least heterozygous for valine at codon 136 (Table 1). With respect to codon 154, no significant differences were found between the groups. Of the 612 sheep genotyped at codon 154 in September 2004 using archived DNA, 10 (1.6%) encoded RH.

TABLE 1. Genotype Results of NDSU Sheep (all tested thru 2002)

	QQAV	QQVV	RRAA	QRAV	QRAA	QQAA	TOTAL
Columbia	4	0	41	8	85	42	180
Hampshire	29	5	11	24	45	32	146
Suffolk	5	0	5	0	16	36	62
Rambouillet	10	1	30	12	49	12	114
Commercial	34	2	66	37	153	44	336
Montadale	1	0	1	0	0	0	2
Dorset	0	0	1	0	0	1	2
TOTAL							842

Scrapie Status Results- Cases of scrapie were reported by NVSL and/or ADRU, ARS, USDA (Table 2). Of the 190 sheep sampled for scrapie status, the genotype results for codon 136 could not be determined for 30 (15.8%) of these animal. Of the 160 remaining samples, 46 were

diagnosed positive for scrapie (2 positives encoding QQ at codon 171 had been excluded from the 48 positive animals because the genotype at codon 136 was unavailable). Of these 44 scrapie positive animals remaining, 43 (97.7%) had valine for one allele at codon 136. Results for the genotype at codon 154 were available for 111 of the negative sheep and 38 of the positive sheep. The frequency of the H at codon 154 was 0% in the positive sheep and 2.7% in the negative sheep.

Table 2. Breed, Genotype and Laboratory Confirmed Scrapie Status of NDSU Sheep, 2002.

	VVQQ		**AVQQ**		**AVRQ**		**AAQQ**		**AAQR**		**AARR**		Breed Summary		Total
	Pos	Neg	*Pos*	Neg	*Pos*	Neg	*Pos*	Neg	*Pos*	Neg	*Pos*	Neg	*Pos*	Neg	
Columbia	*0*	0	*2*	0	*0*	0	*0*	13	*0*	7	*0*	3	*2*	23	25
Hampshire	*5*	0	*18*	1	*1*	2	*1*	8	*0*	4	*0*	0	*24*	15	39
Suffolk	*0*	0	*3*	0	*0*	0	*0*	7	*0*	0	*0*	0	*3*	7	10
Rambouillet	*1*	0	*1*	6	*0*	1	*0*	4	*0*	6	*0*	1	*2*	18	20
Montadale	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*	0	0
Commercial	*2*	0	*11*	18	*0*	1	*0*	25	*0*	7	*0*	1	*13*	52	65
Dorset	*0*	0	*0*	0	*0*	0	*0*	1	*0*	0	*0*	0	*0*	1	1
Genotype Summary	*8*	0	*34*	26	*1*	3	*1*	58	*0*	22	*0*	5	*44*	116	160
Total	8		60		4		59		22		5		160		

Relative Risk- The occurrence of valine at codon 136 conferred susceptibility to scrapie, with the scrapie risk for the homozygote greater than that for a sheep heterozygous for valine (AVQQ) (Relative Risk of 52.3 95% Confidence Interval [CI] 7.417-372.4) (Table 3). The AAQQ genotype confers resistance when compared to the AVQQ and VVQQ genotypes in this flock. The arginine (R) allele offers protection against the risk of scrapie in individuals with one R at codon 171 and encoding a V at codon 136 (AVRQ genotype), with a Relative Risk of .40, 95% CI 0.70-2.23, Odds Ratio 0.21, 95% CI 0.01-2.43).

Table 3. Risk of contracting scrapie with Valine present at codon 136

		Laboratory Confirmed Scrapie Status		
		+	-	Total
Valine present at Codon 136	+	43	29	72
	-	1	87	88
	Total	44	116	160

Relative Risk = 52.3 95% CI 7.417-372.4
Odds Ratio = 129 95% CI 17-979

Ram Genotype- The high incidence of VRQ allelic variant in the flock can be traced to the use of VRQ rams for breeding several years before the diagnosis of scrapie. Genotyping of all rams used prior to the detection of scrapie was not possible, but some rams used by the university had had semen collected and stored in liquid nitrogen. Semen samples (n=21) were therefore

available for genotype determination of these more extensively used rams. Of these 3 (14.29%) had a valine allele present at codon 136. These rams were used extensively beginning in 1992.

Evidence for Lateral Transmission- In two cases, two ewes known to be negative at the time of their natural death had offspring which tested positive, all before their third birthdays. The placenta has been shown to be infective (Race, 1998; Andreoletti, 2002; Pattison et al., 1972) and may serve as a source of environmental contamination with the infectious prion (Ryder, 2004). The occurrence of the infectious prion is dictated by the presence of a conceptus with a susceptible genotype (Tuo, 2001). In the first case (Table 4), the dam was AVQQ, a genotype known to be at increased risk for becoming infected in this flock and her offspring known to have become infected also was AVQQ. In the second example (Table 4), the dam was AVRQ. Although the R at codon 171 has a protective effect, the AVRQ genotype is moderately susceptible to scrapie infection. Her scrapie positive offspring were AVQQ genotype, a highly susceptible genotype. These lambs were not exposed to the disease by their dams, but instead lateral transmission by infectious materials from other positive individuals, most likely when they were lambs, resulted in their scrapie infection.

TABLE 4. Lambs Testing Positive Born to Negative Dams

	Lambing Events		
Ewe ID	**Year A**	**Year B**	**Year C**
Ewe #1(AVQQ)	AVQQ		
Ewe #2 (AVRQ)	AVQQ	AVQQ	Unknown

In addition, several examples exist where both dam and offspring died in the same year from scrapie infection. In the case of a 1999 born dam and her 2001 born offspring, they began showing symptoms of scrapie, after testing third eyelid positive, within seventeen days of one another and were both euthanized on the same day in February 2003. A 1998 born dam and her 2001 born offspring began showing symptoms of the disease 6 months apart and both died in 2003. Both the dams and their offspring had similar lengths of time showing symptoms before their death, suggesting both became infected at the same time, the dam as an adult and the offspring at its time of birth.

Two 1996 born animals in the flock died in 2002 from clinical scrapie. They were VVQQ. This genotype has the greatest risk for contracting scrapie. VV animals at codon 136 are known to have the shortest incubation period and length of survival. In a study of Cheviot sheep, the mean survival time was 907 days for the VV animals (Hunter, 1996). It is therefore unlikely these animals were infected by their dams at their births, but more likely became infected as adults, with the estimated exposure date to be January 2000, based on the known incubation time of VVQQ sheep of approximately two years.

Discussion

Unlike the typical scrapie strain found in the United States, where susceptibility is associated with homozygous glutamine (Q) at codon 171, this flock more closely models European flocks affected by the valine strain of scrapie. In this flock only one positive animal (2.17%) was AA/RR/QQ, whereas 90.91% of the sheep testing positive for scrapie as part of the USDA Scrapie: Ovine Slaughter Surveillance Study 2002-03 were AA/RR/QQ.

This flock had a higher incidence of the valine allele because of a line breeding program utilizing rams heterozygous or homozygous for valine at codon 136. In the USDA Scrapie: Ovine Slaughter Surveillance Study 2002-03, 9.53% of the sample population was found to be at least heterozygous valine at codon 136. A study conducted in Oklahoma found the frequency of the V allele to be 3.99% (DeSilva, 2003). For the present study, NDSU had a significantly greater incidence of valine at 20.4%, resulting in an incidence of scrapie susceptibility above the national average.

In the present study, the occurrence of valine at codon 136 was correlated with being positive for scrapie. One animal positive for scrapie was AAQQ. European reports for Cheviot, Ile de France, Flemish, and Swifter flocks found the AAQQ genotype to be protective, with no homozygous alanine at codon 136 sheep becoming infected. European Swaledale, Texel, and Romanov flocks found the AAQQ genotype to also succumb to scrapie, but remain at a lower risk for acquiring scrapie, with the codon 136 valine associated with a high incidence of scrapie (Review by Baylis, 2004). Two explanations may exist for the occurrence of scrapie in this one animal of AAQQ genotype found in the present study. One possibility is that the flock had two concurrent strains, affecting both alanine and valine sheep. The other possibility is that the contaminated environment (infectious dose) may play a more decisive role in inducing disease in this genotype, with a threshold or dose response effect (Elsen, 1999; Belt, 1995.) AAQQ sheep would thus become infected above a certain level of exposure.

Although the R allele at codon 171 has been found to be protective, the AVRQ genotype remains susceptible to scrapie. However this genotype remains at less risk for scrapie infection than the homozygous QQ at codon 171. The virulence of valine associated scrapie is dependent on the presence and homozygosis at codon 136 for valine and arginine at 171.

In conclusion, this flock had a high incidence of valine associated scrapie and a higher frequency of valine at codon 136 due to the use of rams with valine at codon 136. In addition, lateral transmission by the PrPSc infected placenta played a role in the transmission of the disease in the NDSU flock.

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