

# GENETICS OF SCRAPIE RESISTANCE IN SHEEP

**David L. Thomas**  
**Department of Animal Sciences**  
**University of Wisconsin-Madison**

## **Introduction**

Scrapie is an infectious disease of sheep that affects the central nervous system and is always fatal. Upon necropsy, infected animals will have holes or vacuoles in the tissue of the brain. The disease is classified as a transmissible spongiform encephalopathy (TSE). Some TSE's in other species include bovine spongiform encephalopathy (BSE, Mad Cow Disease), Creutzfeldt-Jakob Disease (CJD) in man, new variant CJD (vCJD) in man thought to result from eating meat from BSE-infected cattle, transmissible mink encephalopathy (TSE), chronic wasting disease (CWD) in some U.S. populations of deer and elk, and feline spongiform ecephalopathy (FSE). Federal and State animal health authorities have considered scrapie a high priority disease for elimination for many years, but the relatively recent evidence that ties vCJD in humans to BSE in cattle has resulted in increased attention on scrapie in sheep.

## **Incidence**

Scrapie was first diagnosed in the U.S. in 1947 in a flock of Suffolk sheep in Michigan that had imported sheep from Canada of U.K. origin for several years. From 1947 through July 2001, scrapie had been diagnosed in over 1,000 flocks, and approximately 1,600 individual cases of natural sheep scrapie and 7 individual cases of natural goat scrapie have been found (Sutton, USDA/APHIS/VS, personal communication). Through 1992, the breed distribution was as follows: 87% Suffolk, 6% Hampshire, and 7% other breeds (Border Leicester, Cheviot, North Country Cheviot, Corriedale, Cotswold, Dorset, Finnsheep, Merino, Montadale, Rambouillet, Shropshire, and Southdown) and crosses (Detwiler et al., 1996; Wineland et al., 1998, USDA, 1999). While 1,600 appears to be a large number of cases of sheep scrapie, it actually is not since it represents an average of only about 30 cases per year. Relative to most other sheep diseases, the incidence of scrapie is quite low.

## **Clinical signs**

Early signs of scrapie include subtle changes in behavior and temperament. These changes may be followed by scratching and rubbing against fixed objects. Other signs are loss of coordination, weight loss despite a good appetite, biting of feet and limbs, and lip smacking. Gait abnormalities such as high-stepping of the forelegs, hopping like a rabbit, and swaying of the back end may be seen. Signs or effects of scrapie usually do not appear until 2 to 5 years after an animal is infected. Sheep may live 1 to 6 months (sometimes longer) after the onset of clinical signs (USDA, 1999).

Infected animals show no immune response to the infective agent as is the case with viral and bacterial diseases. There is currently no live-animal test to determine if an animal is infected.

This is a major area of research, and some promising live-animal tests are in the development and evaluation stages.

The scrapie agent is thought to be spread most often from ewe to offspring and to other lambs in contemporary lambing groups through contact of lambs with the placenta and placental fluids of infected ewes (USDA, 1999). Lateral transmission from infected rams to ewes and lambs, from infected ewes to other ewes, and from an infected environment to adult animals are not thought to be major routes of transmission; although there are documented cases of scrapie where such routes of transmission are thought to have occurred.

There is not agreement among scientists on the nature of the infectious agent. However, infected animals have large amounts of an abnormal type of prion protein (denoted as PrP<sup>Sc</sup>), especially in the central nervous system tissues of the brain and spinal cord. Scrapie-free animals have normal prion protein (PrP<sup>C</sup>) and no PrP<sup>Sc</sup>. Some feel that PrP<sup>Sc</sup> itself is the infectious agent (Prusiner, 1998) while others feel that PrP<sup>Sc</sup> is the result of another infectious agent. However, scientific evidence increasingly supports the theory of PrP<sup>Sc</sup> itself as the infectious agent. Dr. Stanley B. Prusiner, University of California, San Francisco was awarded the 1997 Nobel Prize in Physiology or Medicine for his work on the infectious nature of prions. The infectious agent is extremely resistant to heat and to normal sterilization procedures.

## **Prion protein**

Prion protein is a constituent of normal mammalian cells ((Prusiner, 1998). Sheep prion protein is a protein of 256 amino acids in length. There is some variation among sheep in the particular amino acids that comprise their prion protein. However, the PrP<sup>Sc</sup> found in a scrapie-infected sheep is of the same amino acid sequence as some or all the normal prion protein (PrP<sup>C</sup>) found in that same sheep. The difference between PrP<sup>Sc</sup> and PrP<sup>C</sup> is in its molecular shape. The prion infectious agent theory contends that PrP<sup>Sc</sup> infects a normal sheep and serves as a template to change the normal shape of PrP<sup>C</sup> to the infective shape of PrP<sup>Sc</sup>.

Of the 256 locations for amino acids along the sheep prion protein molecule, amino acid changes in at least three of these locations (amino acids number 136, 154, and 171) have been shown to confer increased or decreased susceptibility to scrapie (Goldmann et al., 1994; Westaway et al., 1994; Belt et al., 1995; Clouscard et al., 1995; Ikeda et al. 1995; Hunter et al., 1997b; Hunter et al., 1997a; Elsen et al., 1999; Thorgeirsdottir et al., 1999; Tranulis et al., 1999). Each particular amino acid is specified in the prion protein gene by a group of three nucleotide bases known as a codon. Therefore, these three important locations are referred to as codon 136, codon 154, and codon 171 on the prion protein gene.

The most common amino acids coded for at these three codons are: alanine (A) or valine (V) at codon 136, arginine (R) or histidine (H) at codon 154, and glutamine (Q) or Arginine (R) at codon 171. Since genes occur in pairs, sheep have two prion protein genes. Each gene can be the same in which case the sheep will produce only one type of prion protein. If the genes are different, then two types of prion protein are produced. Just considering these three codons, a population of sheep theoretically can contain individuals with one of 27 different prion protein

genotypes (Table 1). Not all 27 genotypes have been documented, so some may not occur in nature.

Table 1. Possible genotypes for the sheep prion protein at codons 136, 154, and 171 and a scrapie resistance index for each genotype			
Codons			Scrapie resistance index for U.S. sheep <sup>a</sup>
136	154	171	
AA	RR	QQ	3
AA	RR	QR	9
AA	RR	RR	13
AA	RH	QQ	3
AA	RH	QR	9
AA	RH	RR	13
AA	HH	QQ	3
AA	HH	QR	9
AA	HH	RR	13
AV	RR	QQ	1
AV	RR	QR	7
AV	RR	RR	11
AV	RH	QQ	1
AV	RH	QR	7
AV	RH	RR	11
AV	HH	QQ	1
AV	HH	QR	7
AV	HH	RR	11
VV	RR	QQ	0
VV	RR	QR	6
VV	RR	RR	10
VV	RH	QQ	0
VV	RH	QR	6
VV	RH	RR	10
VV	HH	QQ	0
VV	HH	QR	6
VV	HH	RR	10
<sup>a</sup> Index is sum of numeric value assigned for each individual codon genotype: codon 136: AA=3, AV=1, VV=0; codon 154: RR=RH=HH=0; codon 171: RR=10, RQ=6, QQ=0. Higher values indicate greater expected resistance.			

### Genetic susceptibility or resistance

Genetic resistance to scrapie depends not only on the prion genotype of the sheep but also on the strain of scrapie present. Some genotypes found to be resistant to one strain of scrapie have been shown to be susceptible to another strain. It appears that there are differences between the U.S. and Europe in the major strains of scrapie that are present. The fact that the U.S. is free of certain scrapie strains is good justification for the stringent regulations for importation of sheep and goats into the U.S. from countries with scrapie.

**Codon 136.** Sheep homozygous for alanine (AA) have been shown to be more resistant to scrapie than sheep homozygous for valine (VV) or heterozygous (AV) in European studies. While a lower incidence of scrapie is anticipated in AA sheep in Europe than in the other genotypes, some AA sheep still have been diagnosed with scrapie. AA sheep are not 100% resistant to scrapie. In the U.S., amino acid changes at codon 136 appear to be less important to scrapie susceptibility than in Europe. This may be due to the different strains of scrapie found in the two regions.

**Codon 154.** Of the three important codons, amino acid changes at 154 appear to have a slightly less dramatic effect on scrapie susceptibility than do the other two, and the susceptible genotypes are not consistent across studies. In some studies, sheep with the arginine (R) allele have a lowered incidence of scrapie, and in other studies, sheep with the histidine (H) allele have a lowered incidence. At the present time, it appears that screening sheep on the basis of codon 154 genotype has little value in increasing resistance to scrapie.

**Codon 171.** Amino acid changes at codon 171 have a large effect on scrapie susceptibility in sheep in both Europe and the U.S. Virtually no sheep homozygous for arginine (RR) have been identified with scrapie. The one exception is a single RR Suffolk in Japan that was diagnosed with scrapie (Ikeda et al., 1995). The frequency of heterozygous (QR) sheep also is very low among scrapie-infected sheep. However, the frequency of sheep homozygous for glutamine (QQ) is very high among scrapie-infected sheep.

The last column of Table 1 presents a very crude scrapie resistance index for each genotype in U.S. sheep. The higher the number, the greater the expected resistance to scrapie. The index is weighted heavily in favor of RR and QR genotypes at codon 171, less heavily in favor of the AA genotype at codon 136, and neutral with respect to the genotypes at codon 154.

### **Susceptibility versus scrapie-infected**

It must be remembered that a susceptible genotype like QQ at codon 171 does not imply that the animal is scrapie-infected. Scrapie is not a genetic disease. Scrapie is not caused by a particular genotype. An infectious agent causes scrapie. In order to have scrapie, both an infectious agent and a susceptible genotype need to be present. For example, Australia and New Zealand are scrapie-free, but Suffolk, Cheviot, Merino, and Poll Dorset sheep of susceptible genotypes are found in these countries (Hunter and Cairns, 1998). There is no scrapie in these countries because the scrapie agent is not present.

Likewise, susceptible genotypes are found in many breeds of sheep in the U.S., but almost 90% of the scrapie cases are in the Suffolk breed. This may be due to the major mode of transmission of the disease from an infected ewe to her newborn lambs. Scrapie was brought into the U.S. in Suffolk sheep. A possible scenario that followed was that infected Suffolk ewes infected their lambs, and infected females from infected ewes continued to pass the disease to their lambs. However, infected Suffolk rams would not tend to infect the ewes they were mated to or their lambs, so infected Suffolk rams mated to ewes of a different breed, as is common in crossbreeding systems in the U.S., would not tend to pass scrapie onto that breed.

While scrapie is primarily a disease of Suffolk sheep in the U.S., the disease is found in the Suffolk, Cheviot, Swaledale, Bleu du Maine, Herdwick, Poll Dorset, Shetland, and Soay breeds in the U.K. (Hunter et al., 1997ab), Texel breed in The Netherlands (Belt et al., 1995), Suffolk and Corriedale breeds in Japan (Ikeda et al., 1995), Romanov and Lacaune breeds in France (Clouscard et al., 1995; Elsen et al., 1999), Rygja breed in Norway (Tranulis et al., 1999), and Icelandic breed in Iceland (Thorgeirsdottir et al., 1999). This is not an exhaustive list of sheep breeds or countries with scrapie, but a sample to indicate that scrapie is found in several breeds. A discussion of theories on how scrapie initially becomes established in a breed of sheep would be interesting.

### **Breeding for scrapie resistance in the U.S.**

Selection for resistant prion protein genotypes should be a priority in breeds that have the highest incidence of scrapie in the U.S., i.e. Suffolks and, perhaps, other blackfaced breeds that

have been “improved” by illicit crossing with the Suffolk. Breeders of other breeds that have a very high frequency of susceptible prion protein genes, and no or a very low incidence of scrapie, also may wish to increase the amount of selection emphasis placed on resistant genes in order to safeguard their breed against the effects of possible future exposure to the scrapie infectious agent. With the current strain(s) of scrapie present in the U.S., selection for the arginine (R) allele at codon 171 appears to be sufficient.

A flock where every ewe is of the susceptible genotype QQ can be converted to a flock of QR and RR resistant ewes fairly quickly by simply purchasing and using only RR rams. With a normal ewe replacement rate of 20% per year, over 2/3 of the ewe flock will be of the QR or RR genotypes after five years of RR ram use; even with no selection of ewes or ewe lambs on prion protein genotype. Conversion of the flock to QR and RR ewes will be even faster if, in addition to using RR rams, replacement ewe lambs are preferentially selected from QR or RR dams and if QQ ewes are preferentially culled.

### **Testing laboratories**

A DNA test is available commercially for prion protein genotyping. Generally, DNA is extracted from white blood cells from a sample of the sheep’s blood collected in purple top (EDTA) blood tubes. The following laboratories have been approved by USDA/APHIS to conduct DNA typing for official tests. Contact any of these labs for blood collection and submission procedures and cost.

Biogenetic Services, Inc.  
801, 32<sup>nd</sup> Avenue  
Brookings, SD 57006  
Contact: Alex Kahler  
Phone: 605-697-8500  
Fax: 605-697-8507  
Web: [www.biogeneticservices.com](http://www.biogeneticservices.com)

BioServe Biotechnologies, Ltd.  
1050 West Street  
Laurel, MD 20707  
Contact: Dr. Terri Lehman  
Phone: 301-470-3362  
Fax: 301-470-2333  
Web: [www.bioserve.com](http://www.bioserve.com)

Diagnostic Center for Population  
and Animal Health  
Michigan State University  
Room A45, Veterinary Medical Center  
E. Lansing, MI 48824  
Contact: Roger Maes, DVM, PhD  
Annabel Wise, DVM, PhD  
Mr. Dan Taylor, MS  
Phone: 517-353-2296  
Fax: 517-353-4426  
Web: <http://www.dcpah.msu.edu>

HAP Typing Facility  
Genaissance Pharmaceuticals  
Five Science Park  
New Haven, CT 06511  
Contact: Jeffrey M. Otto, Ph.D  
Phone: 203-786-3546  
Fax: 203-786-3699  
Web: <http://www.genaissance.com>

GeneCheck, Inc.  
1629 Blue Spruce Drive  
Suite 106  
Ft. Collins, CO 80524  
Contact: Ms. Tresea Mulqueen  
Phone: 800-822-6740  
Fax: 970-472-9956  
Web: [www.genecheck.com](http://www.genecheck.com)

GenMARK  
(Vita-Tech Laboratories, LLC)  
1825 Infinity Drive  
DeForest, WI 53532  
Contact: Ms. Lynette Childs  
Phone: 608-846-0580  
Toll-Free: 877-766-3446

Fax: 608-846-0584  
Web: [www.genmarkag.com](http://www.genmarkag.com)  
GeneSeek, Inc.  
4711 Innovation Dr.  
Lincoln, NE 68521  
Contact: Mr. Abe Oommen  
Phone: 402-435-0665  
Fax: 402-435-0664  
Web: [www.geneseek.com](http://www.geneseek.com)

Veterinary Genetics Laboratory  
School of Veterinary Medicine  
University of California  
One Shields Avenue  
Davis, CA 95616-8744  
Contact: Customer Service  
Phone: 530-752-7383  
Fax: 530-752-3556  
Web: [www.vgl.ucdavis.edu](http://www.vgl.ucdavis.edu)

Some states have cost-sharing programs or free DNA testing of flock rams for prion codon 171. Wisconsin had such a program funded by USDA in 2004 and will offer it again in 2005. Special submission forms for the Wisconsin program can be requested from Dr. Don O'Connor, Wisconsin Department of Agriculture, Trade and Consumer Protection, 800-572-8981 (toll free), 608-224-4882 (phone), or 608-224-4871 (fax). Sheep producers in other states should contact their State Department of Agriculture to see if a similar program exists in their state.

## Summary

Sheep scrapie is found at a very low level in the U.S. However, human health concerns surround all TSE's in animals because of the link between BSE (Mad Cow Disease) and human vCJD. Therefore, it is important that the U.S. sheep industry use all the tools available to rid itself of scrapie. The particular prion protein gene that codes for the amino acid arginine (R) at codon 171 has been shown to impart a high degree of resistance to scrapie. Since scrapie in the U.S. is primarily a disease of the Suffolk and "Suffolk improved" breeds, selection within these breeds for "R" at codon 171 should result in an even lower incidence of scrapie in the U.S.

The USDA/APHIS website on scrapie <<http://www.aphis.usda.gov/vs/naahps/scrapie/>> is a very good site for additional information on the disease, control programs, and DNA testing.

## References

Belt, P. B. G. M., I. H. Muileman, B. E. C. Schreuder, J. B. Ruijter, A. L. J. Gielkens, and M. A. Smits. 1995. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *J General Virology* 76:509-517.

- Cloucard, C., P. Beaudry, J. M. Elsen, D. Milan, M. Dussaucy, C. Bounneau, F. Schelcher, J. Chatelain, J. M. Launay, and J. L. Laplanche. 1995. Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. *J. General Virology* 76:2097-2101.
- Detwiler, L. A., A. L. Jenny, R. Rubenstein, and N. E. Wineland. 1996. Scrapie: A review. *Sheep & Goat Res. J.* 12(3):111-131.
- Elsen, J. M., Y. Aigues, F. Schelcher, V. Ducrocq, O. Andreoletti, F. Eychenne, J. V. Ttien Khang, J. P. Poivey, F. Lantier, and J. L. Laplanche. 1999. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. *Archives Virology* 144:431-445.
- Goldmann, W., N. Hunter, G. Smith, J. Foster, and J. Hope. 1994. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J. General Virology* 75:989-995.
- Hunter, N and D. Cairns. 1998. Scrapie-free Merino and Poll Dorset sheep from Australia and New Zealand have normal frequencies of scrapie-susceptible PrP genotypes.
- Hunter, N., W. Goldmann, J. D. Foster, D. Cairns, and G. Smith. 1997a. Natural scrapie and PrP genotype: case-control studies in British sheep. *Vet Rec.* 141:137-140.
- Hunter, N., L. Moore, B. D. Hosie, W. S. Dingwall, and A. Greig. 1997b. Association between natural scrapie and PrP genotype in a flock of Suffolk sheep in Scotland. *Vet. Rec.* 140:59-63.
- Ikeda, T., M. Horiuchi, N. Ishiguro, Y. Muramatsu, G. D. Kai-Uwe, and M. Shinagawa. 1995. Amino acid polymorphisms of PrP with reference to onset of scrapie in Suffolk and Corriedale sheep in Japan. *J. General Virology* 76:2577-2581.
- Prusiner, S. B. 1998. Prions. *Proc. Natl. Acad. Sci.* 95:13363-13383.
- Thorgeirsdottir, S., S. Sigurdarson, H. M. Thorisson, G. Georgsson, and A. Palsdottir. 1999. PrP gene polymorphism and natural scrapie in Icelandic sheep. *J. General Virology* 80:2527-2534.
- Tranulis, M. A., A. Osland, B. Bratberg, and M. J. Ulvund. 1999. Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway. *J. General Virology* 80:1073-1077.
- USDA. 1999. Scrapie factsheet. Online at: <http://www.aphis.usda.gov/oa/pubs/fsscrapie.pdf>.
- Westaway, D., V. Zuliani, C. M. Cooper, M. Da Costa, S. Neuman, A. L. Jenny, L. Detwiler, and S. B. Prusiner. 1994. Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. *Genes & Development* 8:959-969.
- Wineland, N. E., L. A. Detwiler, and M. D. Salman. 1998. Epidemiologic analysis of reported scrapie in sheep in the United States: 1,117 cases (1947-1992). *J. American Vet. Med. Assoc.* 212:713-718.