GENE CHECK, INC.

The Genetics of Scrapie

Bob Wagner

Scrapie is an infectious disease of sheep in which the infectious particle appears to be a particular form of a protein molecule found in normal, healthy sheep(1). The infectious particle (scrapie prion) differs from the normal protein only structurally (the way the protein is folded) and altered prion proteins can apparently induce normal proteins to undergo the structural change to become scrapie prions. Therefore, it might be expected that variants of the prion protein could have differing levels of susceptibility to scrapie, based exclusively on the ease with which a normal prion converts to a scrapie prion.

In fact, current experimental evidence strongly suggests that there are prion protein forms which do not undergo the structural transformation to scrapie prions (although it cannot be positively excluded that they do undergo the structural change, although much more slowly than other protein forms). These prion proteins differ from those which easily convert to become scrapie prions by single amino acid substitutions (amino acids are the individual building blocks of proteins). For example, an arginine (R) at amino acid 171 of the prion protein appears to prevent the prion molecule from undergoing the structural change associated with strain C scrapie. In a similar manner, an alanine (A) at amino acid 136 appears to prevent the prion molecule from undergoing the structural change associated with strain A scrapie.

All proteins are coded for by genes, which are stretches of DNA (the genetic material of all higher organisms). Protein variants are coded for by variant genes, known as alleles. Thus the arginine containing variant at codon 171 (a codon is a stretch of DNA which codes for a single amino acid) can be said to result from an "R" allele of the prion gene. Other alleles known at codon 171 include "Q", which codes for glutamine and "H", which is relatively rare and codes for histidine. Both "Q" and "H" alleles are known to produce prion proteins susceptible to conversion to scrapie prions and are frequently referred to as susceptible alleles. At codon 136, the susceptible allele is "V", which codes for valine. Only "A" and "V" alleles are known at codon 136.

Other alleles have been discovered in sheep prion genes, although none has been positively shown to confer susceptibility or resistance to the structural alterations leading to the formation of scrapie prions.

Sheep, and all mammals, are diploid organisms, which means that their cells contain two copies of each chromosome and, therefore, two copies of each gene(2). Thus a sheep will have two copies of the prion gene in each cell. These copies may be the same or different alleles, but the same copies will be present in each cell of the animal. Thus, a sheep may be characterized with respect to its genotype at a single codon. For example, a sheep may be Q/Q at codon 171, which means that both copies of its prion gene are of the "Q" allele at codon 171. Similarly, a Q/R

sheep has one copy of the "Q" allele and one copy of the "R" allele.

A considerable body of evidence exists indicating that both Q/R and R/R sheep do not (or at least do not readily) develop clinical scrapie. In contrast, the Q/Q genotype is associated with scrapie susceptibility both in experimental studies and in naturally occurring scrapie. Because Q/Q sheep can develop scrapie, it is clear that they can be carriers of scrapie, that is, they can produce infectious particles (prions) which may cause scrapie in other sheep. However, it is important to note what is meant by carrier of scrapie. A sheep with a Q/Q genotype is not guaranteed to be a carrier of scrapie any more than a Q/Q sheep is guaranteed to develop scrapie. To be a carrier of scrapie, a sheep must be exposed to scrapie prions, presumably by being exposed to scrapie. Scrapie is not generally considered to be a genetic disease, but rather an infectious disease with susceptibility determined genetically. Therefore a Q/Q sheep is susceptible to scrapie and a potential carrier. Nothing more. Until we have some method of doing a live diagnosis, nothing at all can be assumed regarding the fate or infectivity of Q/Q (or any other) sheep.

The currently available experimental data do not provide evidence regarding the potential carrier status of either Q/R or R/R genotypes. Thus it is possible that a Q/R sheep may not develop scrapie and yet still infect a Q/Q sheep, which is much more susceptible to scrapie. This might be possible because half of a Q/R sheep's prion proteins contain a "Q" at codon 171 and are, therefore, able to convert to a scrapie prions if they encounter other scrapie prions. The current prion based models for scrapie do not easily accommodate a carrier status for R/R genotypes, unless conversion of the "R" allele derived prion protein to a scrapie prion occurs at such a low frequency or so slowly that the animals do not develop clinical scrapie, but are infectious.

Perhaps at this point some comment on the infectivity of scrapie is in order. Scrapie is not a highly infectious disease. In feeding experiments in which sheep are fed material known to contain scrapie prions it has been found that not all susceptible (Q/Q) sheep develop scrapie. It may be that an extremely large dose of altered prions is required to induce scrapie in adult sheep. (It should be noted that intracerebral injections of scrapie prions into Q/Q sheep appears to be 100% effective in inducing scrapie. However, Q/R and R/R sheep do not develop clinical scrapie even under these conditions.) It is possible that newborn sheep, particularly by virtue of the enhanced protein absorbing capacity of their digestive systems, may be extremely sensitive to prion proteins.

Regardless of the potential carrier status of Q/R and R/R sheep, the current evidence strongly indicates that a flock of R/R sheep will not have clinical scrapie. It makes sense, therefore, to breed for R/R sheep. However, there are many questions that need answers before a producer can embark on such a selection program:

1. Are R/R sheep available today in sufficient numbers and with sufficiently strong phenotypes to allow their widespread use in purebred and commercial flocks?

Not yet, particularly when considering blackface sheep(3). The US blackface sheep population currently contains only about 10% R/R animals and there is probably not sufficient phenotype diversity and quality in this population to meet current needs.

2. Is the scarcity of R/R sheep a consequence of some negative phenotypes associated with the R/R genotype?

Almost certainly not. A genetic analysis of US sheep genotypes reveals no obvious selection against R/R genotypes. In addition, British sheep, which are the ancestors of US Suffolks are almost 50% R/R. It appears that the predominance of Q/Q in American Suffolks is not the result of selection, but rather the unfortunate result of a "founder effect", in which the original Suffolks brought to the US had an over representation of Q/Q genotypes, purely by chance.

3. If there are not sufficient R/R sheep available today, is genotype selection still practical?

Definitely. Given low susceptibility of Q/R sheep to clinical scrapie, it makes sense to move gradually to R/R sheep by going through Q/R. Approximately 50% of US Suffolks are Q/R. At least 50% of the offspring of Q/R sheep will also be Q/R. 25% of the offspring of a Q/R x Q/R mating will be R/R. Thus, if a Q/R ram is bred to 100 randomly selected ewes, and a 150% lamb crop is obtained, approximately 19 R/R lambs will be produced(4). Even if the flock has the unfortunate bad luck to be 100% Q/Q, the first breeding with a Q/R ram will produce approximately 75 Q/R lambs.

4. Will we sacrifice important phenotypic traits while selecting for R/R genotypes?

No. The beauty of the scrapie situation with respect to genetics is that it provides sheep producers the opportunity to select for a genotype rather than a phenotype. This has not been possible before. Therefore, a producer should identify a phenotype with which he is satisfied and then select for Q/R (or R/R) genotypes among the group of sheep with the desired phenotype. Because the genetic data reveal no selection against any codon 171 genotypes, all genotypes should be represented among all phenotypes. This is not to say that all lines will contain all genotypes. A given line may be subject to the same sort of founder effects as appear to have occurred with the first American Suffolks. It is, therefore, important to distinguish phenotype from line.

5. Testing is expensive. Do we really need to test all of our sheep in the beginning?

Probably not. Initially it is probably sufficient to test stud rams and assure that they are at least Q/R. When the flock is using only R/R rams, it makes sense to test stud ewes as well in order to avoid the need to test all lambs. Remember, all lambs out of R/R x R/R matings will be R/R. Once a flock is entirely R/R, it will be unnecessary to test at all. Two exceptions exist to this advice. First, producers in a hurry will make faster progress toward an R/R flock by testing all their sheep from which they will keep replacements. Second, producers who already have a lot of "R" in their flocks (as determined from testing stud rams) may save money in the long run by testing the entire flock.

6. If Q/R sheep are not susceptible to scrapie and much more common than R/R sheep, why not select for Q/R only?

Several reasons. First, Q/R sheep crossed with other Q/R sheep will produce offspring of different possible genotypes such that the lambs will require testing (1/4 of the offspring will be Q/Q). Second, although it is clear that Q/R sheep are less susceptible to scrapie than Q/Q sheep, it is possible that they may be slightly more susceptible than R/R sheep. Third, until we have good data regarding carrier potential, it may be safest to assume that Q/R sheep have the potential to be carriers of scrapie and are more likely than R/R sheep to have that potential.

7. Are there any risks to this program?

There is one slight, but preventable risk that deserves mentioning. We have been concerned in this discussion almost exclusively with codon 171 genotyping. Strain C scrapie (which is associated with codon 171 genotype) is the predominant if not exclusive form of scrapie in the US. However, there is evidence that V/V Q/Q(5) sheep are extremely susceptible to scrapie. This is a genotype that should be avoided at all costs. There has been no example reported of a "V" allele on the same chromosome as an "R" allele. It is most likely that "R" (codon 171) alleles arose during evolution on "A" (codon 136) chromosomes. If so, it is highly unlikely that "V" will ever be found on "R" chromosomes. Therefore, all R/R sheep should also be A/A. Fortunately the "V" allele is rare in Suffolks. Nonetheless, in the course of building an R/R flock by using Q/R sheep it is possible the a producer may have a V/A Q/R sheep. If two such sheep are bred, 25% of their offspring will be V/V Q/Q and prime candidates to develop scrapie, if exposed. Therefore, it may be advisable to test all Q/R sheep (or at least Q/R stud rams) to be certain they are A/A at codon 136.

Footnotes

1. There have been reports claiming to demonstrate that scrapie may be transmitted by some virus-like entity distinct from the altered prion protein. There is not widespread support for this theory and the data allow other interpretations. In any event, susceptibility to clinical scrapie appears to be determined genetically by the state of the prion gene.

2. For purposes of this discussion, we will ignore sex chromosomes

3. Much of the discussion regarding genotype frequencies and selection schemes will be concerned with blackface sheep, primarily Suffolks, as most of the scrapie in the US is found in Suffolks and most of the genetic data regarding susceptibility in the US has been obtained with Suffolks.

4. Assuming 50% of the ewes are Q/Q and 50% are Q/R. The number is higher if 10% of the ewes are assumed to be R/R.

5. V/V at codon 136 and Q/Q at codon 171.