



# GENE CHECK, INC.

## A Live Animal Test for Scrapie

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Scrapie and government scrapie policies are currently having a major impact on the sheep industry. The recent pattern of flock depopulations by APHIS and several states threaten the very survival of the sheep industry in the US and have resulted in a concerted outcry from all sides of our industry. One of the calls that we hear and read frequently is for our government to spend a larger fraction of the millions now being spent on scrapie for research to develop a “live animal test” for scrapie. These calls are certainly well-intentioned, but are misdirected. The reality is that we already have a nearly perfect live animal test for scrapie that meets all the needs of the sheep industry.

Why the confusion? As with so much of our misunderstandings surrounding scrapie, the problem is that we try to make scrapie more like diseases with which we are familiar. For diseases caused by bacteria and viruses, relatively simple and inexpensive tests can be and have been developed to detect the organism in an infected animal. We are all familiar with the test for *Brucella ovis* (*B. ovis*). A test to detect this bacterium is required for virtually all interstate movement of rams over 6 months old. A blood sample is taken and a test performed to detect antibodies against the bacteria. (animals make antibodies to repel infections by foreign organisms.) Tests to detect antibodies are relatively inexpensive and easy to perform.

Scrapie is NOT caused by a bacteria or a virus or any other “conventional” infectious agent. Scrapie is caused by proteins (scrapie prions) which are actually made by the infected animal. Although the process is started by an “infecting” prion from another animal, the foreign prion recruits other prions by converting normal prion proteins in the infected animal. Although the process can take two to five years, it is ultimately the infected sheep’s own proteins that cause scrapie and kill the animal. Because these proteins are recognized by the animal as “self,” no antibodies are made.

However, it is possible to detect prions in an affected sheep. In fact, the current best method for post mortem scrapie diagnosis is immunohistochemistry (IHC), a big word for a method which uses antibodies (made from animals other than sheep) to detect prions in brain sections. Unfortunately, this accurate and reliable test method is certainly not a live animal test since it requires brain samples. Most of us are also familiar with third eyelid testing, which is a live animal test, although not the kind of test the sheep industry really can use. Third eyelid testing takes advantage of the fact that prions tend to accumulate in lymph tissue. The third eyelid (nictating membrane) of sheep contains two small lymph glands which can be removed without permanent harm to the animal and used in IHC tests.

So why isn't third eyelid testing the answer to the scrapie problem? Primarily because a negative result is not an indication that the sheep will never get scrapie or even that it has not already been infected. Remember, scrapie takes a long time to develop. Third eyelid testing is only really good at detecting positives 10 to 14 months after infection. If a sheep was exposed to scrapie at birth, it would test negative in the third eyelid test until it was nearly a yearling, even if it was destined to die of scrapie before its fifth birthday. In other words, positives in the third eyelid test really are positive, but negatives don't mean anything. What the sheep industry wants is a test where negative means that an animal is free from scrapie. Tests that depend on prion detection are unlikely ever to be sufficiently sensitive to accomplish that goal, particularly immediately or soon after exposure.

The good news is that there is a test available today that guarantees that a sheep does not have scrapie and will not acquire scrapie, even if exposed. That test is the codon 171 test. The codon 171 test is a DNA test that determines the amino acid (building block of protein) in the 171<sup>st</sup> position of the prion protein. The test determines the genotype of the codon ( a short stretch of DNA that codes for a single amino acid) that codes for the 171<sup>st</sup> amino acid. If a sheep has an "R" amino acid at position 171 it is "negative" for scrapie, which means that it does not have and will never get scrapie. "R" actually stands for the amino acid arginine, but it is easiest to think of it as standing for resistance. Since sheep, like humans, have chromosomes in pairs (one from the mother and one from the father) they may be RR (both chromosomes with the resistant gene), QR (one chromosome with the resistant gene) and QQ (sensitive to scrapie).

The best news of all is that only one "R" gene is necessary to confer virtually complete resistance. No QR or RR sheep has ever been confirmed to have scrapie in the U.S. and no QR sheep has ever tested positive in a third eyelid test. Recent work examining placental tissue from QR lambs whose dams (QQ) are affected with scrapie indicates that QR sheep cannot be carriers. Relative to third eyelid testing, DNA testing is simple (it uses only a small blood sample) and inexpensive and DNA does not change. A lamb that is RR will always be RR and will, therefore, always be negative for scrapie.

Perhaps instead of calling for more research to develop more tests, we in the sheep industry should be calling for implementation of rational scrapie programs based on the science we already have. If a small fraction of the money spent to date on scrapie programs could instead have been used to subsidize producers to purchase RR rams, we could be well on our way to eradicating scrapie. Remember, every lamb from an RR ram will be at least QR and, therefore, resistant to scrapie. Without large expenditures for testing, it would be possible, perhaps by requiring producers to use only RR rams, to eliminate scrapie in a few years. For example, if a commercial sheep producer used only RR rams to sire replacement ewes, the entire flock would be at least QR (and free of scrapie) when the last QQ ewe left the flock (generally at around 5 years old in most commercial operations). Start using only RR rams in 2002 and be completely scrapie free in 2007. All without testing a single female. That is powerful technology.

By the way, using QR rams can accomplish the same result, but you will need to test the lambs and send the QQs to market. Also, QR rams can be used as terminal sires without testing the lambs, provided that they

are slaughtered before they are one year old.

We have the ability, thanks to science and DNA testing, to eliminate scrapie without killing a single ewe. Even QQ ewes can produce resistant (QR) lambs. No other species is known to have such “dominant” resistant to transmissible spongiform encephalopathies (TSEs, which include scrapie, CJD, Chronic Wasting Disease, Mad Cow Disease, etc). We should insist on being able to use this powerful “live animal” test to eradicate scrapie without eradicating the sheep industry.