GENE CHECK, INC.

SCRAPIE AND CODON 171

Part 1: Can we eliminate scrapie genetically?

Bob Wagner

Sometimes it feels good to be a sheep breeder. Granted, we have our problems and scrapie is certainly one of them, but sheep are the only species for which it is possible to breed for resistance to spongiform encephalopathy (scrapie, mad cow disease, Creutzfeldt-Jakob disease, etc). In fact, it is entirely possible that scrapie can be eliminated with the genetic tools we now have at our disposal.

Twenty years ago (January 1979) a British scientist named Parry published a paper in the scientific journal Nature entitled "Elimination of natural scrapie in sheep by sire genotype selection". This paper reported the results of more than twenty years of research in which Parry studied flocks with high levels of scrapie and selected for rams whose offspring did not die of scrapie in spite of known exposure. He found such rams (He called them "proven white" or SS; today we call them RR). None of the offspring of these rams showed any signs of scrapie, even if their dams died of scrapie during lactation! A quote from the paper probably says it best:

"1,223 Progeny, 1,167 female and 56 male, of the 18 'proven white' rams were born and reared to adults in 15 flocks. 825 Females and 12 males were observed to 4 yr or older, many to 7 yr, the oldest being 13 yr. **No case of scrapie has occurred in these sheep** [my emphasis], although all 15 flocks contained affected animals during some part of the observation period, 1958-78. Sixty-three progeny were out of dams affected during their gestation or suckling, or manifesting scrapie within 2 yr.; 46 were observed to 4 yr or older, with 25 observed to 7 yr and some to 11 yr; no clinical signs of scrapie were observed. Eight of the oldest animals, which had lived continuously in contact with clinical scrapie, showed no microscopical evidence of scrapie ..."

The work of Parry was good science and gave powerful results. That sheep born of dams that were affected with scrapie during gestation or lactation showed no signs of scrapie in brain sections at 9 - 10 years old indicates the true power of genetics. And remember, this was done at a time when we didn't know much about prions and DNA testing was non-existent.

Why has this remarkable and persuasive work been largely ignored, at least in North America? Probably because Parry drew the wrong conclusion from his results. He felt that the results proved that scrapie was a genetic disease. It's really a shame that he neglected to mention the other possible conclusion that could be drawn from his results, namely that **susceptibility** to scrapie was genetic, because his data clearly prove that there is a genetic element involved with scrapie. Much additional evidence has accumulated over the years to confirm that scrapie susceptibility is genetically controlled and that QR and RR sheep are resistant.

So why isn't everyone doing genetic DNA testing and breeding for RR sheep?

Those opposed to testing and breeding for RR sheep generally express concerns that RR and QR sheep MAY (very strong

emphasis on the "may") have been found to have scrapie and that RR and QR sheep may be carriers of scrapie without getting clinical scrapie themselves.

There has never been a North American sheep of QR or RR genotype confirmed to have scrapie or even to have tested positive in the new live animal (third eyelid) test for scrapie prions. But even if a rare RR or QR sheep is found to test positive, would that invalidate the use of genetics as a tool for the control of scrapie? If a scientist reported that an occasional sheep clearly vaccinated for overeating still got the disease would you stop all vaccination?

As far as carriers are concerned, there is absolutely no scientific evidence that sheep which do not have or will not get scrapie can pass the disease on to other sheep. In fact, a very recent study of a flock of Romanov sheep in France, which studied 1015 exposed animals in a single flock, concluded that carriers were unlikely: "Our finding that animals from healthy dams had significantly lower risk if their dam was resistant rather than susceptible, is a positive argument against the infectivity of healthy genetically resistant carriers." (Elsen, et al. 1999. Archives of Virology **144**:431-445).

DNA testing (genotyping) is a tool. It is a very powerful tool and may even provide for the eradication of scrapie but, in any event, it is a tool we should be using even if it only reduces the incidence of clinical scrapie. The way epidemiology works is that if you reduce the incidence of an infectious disease in the host which produces the infectious agent, you reduce the amount of infectious agent in the environment, which further reduces the incidence of the disease, and so on until, with time and a little luck, the disease is eradicated. Scrapie may be that easy to eradicate. However, it may be necessary to continue to identify and remove from the national sheep herd all animals with clinical scrapie and those which, as may be identified by live animal testing, are going to become clinical.

Why does this difference of opinion with respect to genetics and genetic testing exist at all? It may have most to do with the very nature of the disease itself and the fact that to attempt to approach this disease as we have approached other diseases in the past may not work. The spongiform encephalopathies are an unusual group of diseases. Although there is a small group of diehard scientists who continue to argue for some viral cause, the vast majority of scientists now believes, and the scientific evidence supports, that spongiform encephalopathies are caused by infectious protein particles known as prions or prion proteins. What distinguishes prion infections from the viral and bacterial infections with which we are all familiar is that the infecting particle does not duplicate itself to cause disease (proteins do not replicate), but rather "recruits" other protein molecules from those being manufactured in the cells of the host animal. The process is somewhat analogous to crystal formation where a seed crystal causes other molecules to condense around it to form a large crystal. Infecting prions cause normal prions to convert from their normal structure to the infectious form (also known as scrapie prions). Once converted, the scrapie prions are virtually indestructible and can themselves cause other normal prion proteins to convert.

What is important to note about the process of prion diseases is that it is ultimately the victim's own converted proteins which cause death. Therefore, it has been extremely difficult to detect the disease in a live animal. Other live animal tests with which we are familiar, such as the ELISA test for *B. ovis*, depend on detecting antibodies to the invading agent. Antibodies are produced as a means to ward off the invader and depend upon detecting differences between the invader and the host. In the case of prion infections, the particles that kill are actually host proteins produced by the host and cannot, therefore, be targets for host antibodies.

Recently, tests have been developed which can detect altered (scrapie) prion proteins

and distinguish them from normal proteins. The test examines lymphatic tissue from the third eyelid of living sheep. Preliminary results from third eyelid test studies of high risk (exposed) sheep strongly support the notion that genetics is the way to control scrapie. In other words, no QR or RR sheep has tested positive in these tests. QR sheep out of affected QQ dams also are testing negative.

If, as appears more and more to be the case, genetics is the way to control scrapie, how does genetics work in determining susceptibility to scrapie? Conversion of normal prion proteins to scrapie prions depends on the primary structure of the protein. Primary structure is the linear arrangement of the amino acid subunits that make up every protein (picture a wire strung with beads). Function of a protein depends on its secondary structure, which is the pattern of folding of the string of amino acids (picture coiling or folding the wire strung with beads). Although secondary structure is determined by and dependent upon primary structure, more than one secondary structure may be possible for a given primary structure. Such is clearly the case for the prion protein, where one secondary structure is the normal protein and another (same primary structure) is a scrapie prion. Since primary structure determines secondary structure, it should not be surprising that a change in primary structure can make (or prevent) a change in secondary structure. Such is also the case for the sheep prion protein. Changing the 171st amino acid of the protein (which is coded for by codon 171 of the gene) from a glutamine (Q) or a histidine (H) to an arginine (R) dramatically changes the prion protein's ability to convert to a scrapie prion. In fact, a prion protein with an R at amino acid 171 converts very poorly (if at all) to a scrapie prion.

Some people argue (perhaps correctly, but there is no good scientific evidence) that R-containing prion proteins do convert to scrapie prions, they only do it much more slowly than Q-containing prion proteins, such that, if they lived long enough, RR sheep exposed to scrapie would all get scrapie. But again, the goal of a scrapie eradication program should be to eliminate the infectious agent. If R-containing prion proteins are less likely to become scrapie prions than Q-containing prion proteins (for any reason, including the fact that they convert more slowly), shouldn't we be breeding sheep that do not produce Q-containing prion proteins?

What about carriers? Should we worry? First, as mentioned above, any concern regarding carriers suffers from lack of good scientific evidence and there is now some good evidence against carriers. No one has ever demonstrated that a sheep that will not develop clinical scrapie can transmit the disease. Second, even if carriers were demonstrated, the arguments are again the same: a prion protein that does not convert or converts poorly or slowly is less likely to transmit scrapie (with or without exposure) than a prion protein that converts readily.

Finally, if there were to be spontaneous conversion of prion proteins to scrapie prions, it would occur in the genotypes of prion proteins which convert most easily with exposure, namely Q-containing prion proteins.

Thus, there seems to be good reason for avoiding Q-containing prion proteins, which is the same as saying that we should be breeding our sheep to be RR. (By the way, you do not need to worry about any other codon, including codon 136. If a sheep is RR at codon 171, it will be AA, the good genotype, at codon 136.)