January 14, 2014

American Gelbvieh Association
10900 Dover Street
Westminster, CO 80021

Dear Board of Directors,

As you are aware, we have recently determined that there is an issue with the current DNA-based diagnostic for Contractural Arachnodactyly (CA) when used within certain subpopulations of the Gelbvieh breed; CA is a genetic abnormality that is believed to have originated within the Angus breed. I am writing this letter to formally update you on the current status of our investigation into this matter. Please feel free to distribute this information to the Association's members. To provide an appropriate background for members that may be unaware of the situation, I will start by summarizing what has happened during the past several months, leading to the discovery of this problem. This will be followed by an explanation of what is currently being done to find a solution for Gelbvieh breeders.

It is my understanding that while testing animals for CA, in accordance with Association policies, a couple of animals were genotyped as CAC, yet had no or very limited Angus-influence in their pedigrees. New samples for several of these animals were resubmitted to my laboratory to confirm these results. In all cases, our testing showed that the animals were correctly genotyped as CAC. Therefore, due to the uniqueness of the CA mutation and the diagnostic that was developed based on it, the most likely conclusion was that even with the limited Angus-influence, these animals were likely to be true carriers of the CA mutation potentially originating from a carrier foundation animal within the purebred registry. However, subsequent testing of both ancestors and progeny of these CAC animals revealed additional and very important information. Firstly, CAC animals were detected within fullblood pedigrees that predate the oldest known carrier ancestor in the Angus breed. Secondly, several animals were genotyped as affected (CAA), yet had no physical symptoms of CA disease pathology.

The presence of CAC animals among early fullbloods is important as this information contradicts our current knowledge about the origin of the CA mutation. Genetic mutations occur at a single point in time and are unique. When a mutation occurs, it does so in a single animal and that animal becomes the "founder" within the population. From that point forward, all future carriers are obligated to have a relationship to the founder animal. Furthermore, the probability of the same exact mutation occurring in two different animals of different breeds is infinitesimally small, if not impossible. As mentioned previously, up until these early (e.g., 1972)

fullblood Gelbvieh animals were genotyped, the oldest known carrier animal in the Angus breed was born in 1978. Thus, if these fullblood animals are truly carriers of CA, there is an obligatory relationship between the Angus and Gelbvieh breeds that is not currently documented in the history of either breed. If there is an unknown relationship, this would suggest that the CA mutation would have had to been introduced into the Angus breed from an early Gelbvieh animal. Although this may be possible, it is improbable.

The presence of CAA animals that were phenotypically unaffected is also unexpected. Given the severity of the mutation involved in CA, where a significant portion of the gene involved is deleted, it would be hard to envision how an individual could be homozygous for this mutation and have normal development. Indeed, genotyping of more than 88,000 Angus animals for CA reveals that only four animals have genotyped as CAA, most of which had disease symptoms; in comparison, 17 Gelbvieh animals have genotyped as CAA out of only 2,817 genotyped and appear to be phenotypically normal. There are possible biological explanations as to how this could occur but, they are less likely than a potential problem with the diagnostic itself. When these diagnostics are designed, they are specifically made to protect against false negative results. This is because false negatives are contradictory to applying negative selection pressure on these mutations (i.e., missing a carrier animal translates to higher risk to the population should that animal be heavily used). Due to this process, the most common error associated with a diagnostic is failure to recognize normal alleles, resulting in carriers incorrectly genotyping as homozygous for the mutant allele. This is primarily caused by differences in the DNA sequence between various normal alleles and the mutant allele in the population. Thus, even after validation of the CA diagnostic in several thousand Angus animals, it is possible that a normal version of the chromosome present in Gelbvieh cattle is not properly being recognized by the diagnostic reagents.

Unfortunately, the observations of early fullblood Gelbvieh animals being carriers and CAA genotyped individuals being unaffected do not suggest a single common problem. In the CAC genotyped animals, the diagnostic appears to be accurately detecting both a normal and mutant DNA sequences, whereas in the CAA individuals the diagnostic appears to not recognize at least one variant of a normal allele. Given these observations, we initiated an investigation aimed at better understanding what might be occurring. Firstly, we inquired as to whether or not all CAA individuals had both a sire and dam that were CAC, including DNA verification of their recorded pedigrees. If the diagnostic were failing to recognize a normal allele, we would expect that these CAA genotyped animals would be the result of CAF x CAC matings instead of the expected CAC x CAC matings. Of the eight parentally verified CAA animals investigated thus far, seven are the result of CAC x CAC matings and one the result of a CAF x CAC mating. Although the results from one individual suggest the diagnostic is not detecting a normal allele,

the other seven clearly indicate that the parents are heterozygous (i.e., both normal and mutant alleles are being detected) yet the progeny are CAA. Therefore, the data suggest that these animals are truly homozygous. Based on this information, we sequenced the DNA fragment produced from the CA diagnostic assay that indicates the presence of the mutation. The DNA sequence from each of these CAA animals was also identical to the mutant allele found in Angus cattle. However, due to the sequence complexity of the chromosomal region containing this gene, we inquired if the DNA segment that is deleted in the CA disease-causing allele was truly absent in Gelbvieh cattle, as it is in Angus cattle. Of the seven CAA animals analyzed, all appear to have a complete intact protein-coding sequence. This contradicts the previous data suggesting that they should be phenotypically affected and indicates a likely problem with the CA diagnostic.

Based on these data, we conclude that the CA diagnostic is potentially detecting sequence variation unique to a specific fullblood Gelbvieh lineage. This sequence variation causes the assay, as it is currently designed, to produce a false positive test result and misassignment of both CAC and CAA genotypes within individuals related to particular fullblood ancestors. Thus, in light of these data, the current diagnostic is unable to distinguish between this putative normal allele and the CA mutant allele previously identified in Angus cattle. Until we can provide a solution to this issue, the CA diagnostic should be considered unreliable within specific pedigrees of the Gelbvieh registry. We have designed several additional experiments in an effort to determine exactly what can be changed in the diagnostic and hope to have a solution within the next 30 days.

Sincerely,

Jonathan Beever, PhD
Professor of Genetics