Questions for Dr. Center. Yorkshire Terrier Club of America. Memphis, Oct. 30, 2009

Prior to Dr. Center's seminar, the membership sent in questions they would like answered. Some questions were consolidated as a number were similar. These answers are based on a compilation of notes taken at the seminar by Charlie Farrar, Crystal Messersmith and Lisa Bridgewater.

**BILE ACID TESTING**

1. Please give us a standardized protocol for the Bile Acid test; per fast and post fast. Exact food to feed(name brand), quantity per pound, time frame to wait between blood draws.

   *Collect a bile acid sample before a meal (pre-meal) and 2 hours after a normal meal (post-meal or postprandial). It is no longer recommended to fast for 12 hours before first collection or meal. The same size and type of meal that the dog normally eats is what should be used for the meal. In order to get the best baseline, it is recommended that puppies be tested at 16 weeks and again at 6 months.*

   *The optimal meal is one with the dog's regular food. Stuffing the dog, the dog being stressed or frightened can possibly skew results. The draw can be done one day pre feeding, then the dog can be taken home and fed and another draw done 2 hours post-feeding. It is not imperative it be done the same day.*

2. Bile Acid Testing – Criteria for best draw. Also, I have heard from a number of people that they have had pretty divergent results on multiple draws. What factors are most likely to influence this?

   *Dog should not be stressed prior to draw (see above). Blood should be collected into lithium vacutainers. The blood must be treated gently as rupture of the red blood cells complicates testing. Red color competes with the blue dye color in the assay that measures the concentration of bile acid. Lipemic blood (fatty blood) after the meal increases red blood cell fragility.*
   a. *Collect blood into syringe using a butterfly set up.*
   b. *Remove needle from syringe and remove the top of the vacutainer tube*
   c. *Gently put the blood (roll down side of the glass) into the vacutainer*
   d. *Replace the rubber top on the vacutainer, making sure it seals tightly*
   e. *Centrifuge the sample to separate plasma from red blood cells*
   f. *Put plasma in separate tube before mailing*
   g. *DO NOT USE separator tubes as these are inconsistent in the their ability to keep red blood cells and plasma separate*
   h. *Bile Acid cannot be measured in EDTA blood, so do not collect into purple top tubes*
   i. *Volume of blood necessary for bile acid determination: at least ½ m; (.5 ml) of PLASMA, that means 1 to 1.5 ml blood*

   *Many things can influence the results. A dog that has been car sick or stressed will test differently. Testing older dogs (>4 years) that have a history of illness may disclose high bile acids due to another disease process rather than the PSVA/MVD.*

3. What is the best age to have a puppy /dog Bile Acid tested?

   *16 weeks and again at 6 months.*

4. How accurate is the testing and does age have a factor… (dog vs puppy)?

   *For the most accurate assessment of the dog's possible genetic status it is best to test dogs young. Older*

*dogs (>4 years) can have other liver problems that will be misleading.*
*Dr. Center emphasized that the bile acid test is not diagnostic, ie, it does not diagnose what type of problem a dog has, just that there is likely a problem.*
*She did indicate that in shunt dogs you generally see the following:*
*Bile Acids > 100*
*Red blood cells microcytic*
*Cholesterol Low*
*BUN Low*
*Creatinine Low*
*Glucose, may be Low*
*Ammonium biurates in the urine.*

5. What are the numbers that reflect if a dog/bitch should be bred or not?

*Until the marker is found, Dr. Center said she does not recommend culling any dogs/bitches from breeding programs. Keep in mind that if dogs over the range of >25 post are bred, you are taking the risk of perpetuating the trait and disease. Breeding animals that are over normal levels should have exceptional qualities in order to be considered to be bred.*

6. If you get a high number, should the dog/bitch be tested again?

*Yes, if there were unusual circumstances surrounding the draw or poor collection methods were used. Keep in mind that the rate of stomach emptying after a meal and how fast food moves through the intestines to the area of high bile acid reabsorption in the ileum varies in individual. You however should not be attempting through multiple tests to attain a low reading and consider that reading the accurate one. If that dog is really valuable to your breeding program you can retest at a different lab*

7. It has been rumored that a specific type of diet and /or use of a drug can lower the levels during testing therefore affecting the results. Is this true?

*Won't affect PVSA dogs, could possibly affect MVD dogs.*

8. What are you finding to date on genetic transference, Any thoughts on this at the present time? In pedigree analysis have they seen a pattern?

*The genetic model best fitting the data is trait transmission as an autosomal dominant with incomplete penetrance. Since a few dogs have been identified with vascular malformations not influencing hepatic perfusion, we acknowledge that serum bile acids are unable to correctly phenotype all dogs carrying the PSVA/MVD mutation. Currently, breeders are advised to avoid using dogs with total serum bile acid concentrations >.25uM/L as foundation stock, and to appraise the bile acid status of all puppies produced to identify parents silently carrying the trait.*

*It has been found that two dogs with normal bile acids can produce affected puppies. It is hypothesized that the genetic transmission is polygenetic with a modifier gene or genes, likely autonomic dominant with incomplete penetrance, as stated above. For example the following model:*
*A, B = Dominant*
*a, b = Recessive*

*aAbB = Affected*
*aaBb and Aabb = Unaffected*
*thus aaBb X Aabb can produce an affected puppy*

*It would be suggested that if you breed a pair and the offspring have high bile acids, the breeding should not be repeated.*

9. If a dog/bitch produces a PCVA or MVD puppy and is itself below level – should it definitely be culled from a breeding program?

*Until the marker is found, no definite recommendation can be made. Breeders must keep in mind that ignoring these types of things may perpetuate this disease. Our understanding was that she would not repeat the breeding ie, same parents, but would not exclude from breeding to a dog from other line. She also suggested on #4 to change stud dogs and see what happens.*

10. Are your guidelines re breeding <25 bile acids subject to change based on the circumstances and what would these circumstances be?

*Answered in prevision questions.*

11. Is there an instance where you would recommend using a dog that tests in the higher ranges (over 50)? If so, why?

*She would not recommend this. But as stated previously, if a dog is bred with higher bile acids, the breeder should be aware they may be perpetuating the problem.*

12. Is it clear that both parents have to have to be at least carriers in order to produce these diseases?

*She didn't directly answer this one, but since she is assuming autosomal dominance then it is implied that no – both do not have to be carriers.*

13. Has it been proven that other diseases are linked to these diseases, such as Leggs-Perthes, Umbilical Hernias or other vascular diseases? IF so, which ones?

*No*

14. After all this is known and not known about Liver Shunt – Is a breeding program that has never produced a liver shunt pup and does not bile acid test have any greater a risk of producing one than a breeding program that does bile acid test and only breeds individuals according to certain levels? What data do you have to support your answer?

*Likely. As stated previously, two apparently normal dogs can produce a PSVA or MVD puppy. It is likely the luck of the draw if it has not been produced, as she feels the trait is highly prevalent in the Yorkshire Terrier based on her research. It is her feeling that breeders that do not test are again sticking their heads in the sand. Select Tibetan Spaniel breeders have used the bile acid test to greatly reduce the problems in their lines (by breeding low to low)– after several generations.*

15. What will it mean to a breeder after the genetic marker is found? Should all dogs found to be carrying the gene for liver disease be sterilized? How will it be determined which dogs are breedable?

*With a genetic test, you would be able to determine if a dog is a carrier and make informed breeding decisions, ie how or if the dog should be bred. As stated above, she is not recommended spaying/neutering at this time, as we do not have the genetic marker and could make some irreversible mistakes.*

16. How close is the release of the test for a genetic marker for liver disease?

*Gene mapping has just been started. Hopefully it will be within a year but there are many factors that can change that. Funding is still needed as people are being taken out of the program due to costs.*

17. What additional information or funds are needed for you to be able to find a marker?

*She reported that she has over 3000 samples of Yorkies. Funds however are running low and mapping is just starting.*