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BOXER Update

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DNA DISEASE TESTING

Tools to Guide Breeding Decisions

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BOXER DNA TESTS

AID UNDERSTANDING OF HEART & NEUROLOGIC DISEASES

Boxer breeders are fortunate to have genetic tests for the well-known heart disease arrhythmogenic right ventricular cardiomyopathy (ARVC) and the progressive disorder degenerative myelopathy (DM). It may seem as though it would be easy to selectively breed away from these devastating conditions, but it's not simple.

PHOTO: GAY GLAZBROOK

Both diseases are believed to involve multiple genetic and environmental factors besides the causative gene variant for which their DNA tests screen. Nonetheless they are helpful tools. The American Boxer Club includes both genetic tests in its recommendations for Health Screening for Boxers in a Breeding Program.

ARVC has an autosomal dominant mode of inheritance, meaning affected dogs need only one copy of the deletion mutation in a gene producing striatin, a key binding protein involved with the heart's electrical functioning. The heart muscle disease has variable penetrance in which some Boxers die suddenly following a run of ventricular premature complexes (VPCs) or succumb over time from congestive heart disease, yet other dogs develop a mild, medically manageable case.

Likewise, DM is a complex genetic disease. Although it is an autosomal recessive disorder in which an affected Boxer inherits the superoxide dismutase 1 gene (*SOD1*) mutation from both its sire and dam, the neurodegenerative disease has incomplete penetrance. Thus, only some affected at-risk dogs develop the disease, though all Boxers with the mutation pass the mutant alleles to their offspring.

As both diseases develop in adult dogs — ARVC occurs on average at 6 years of age and DM at 9 years of age — it is possible that a Boxer has already been bred when a gene mutation is recognized. If dogs are not genetically tested early in life, clinical signs of heart disease or the neurodegenerative disorder may trigger the discovery.

Rhoda Goselin-Brouillette and Tracy Hendrickson of Broken Arrow, Oklahoma, who breed Boxers under the Sunchase prefix, are diligent about following good breeding practices. They rank ARVC and DM high on their list of health conditions to try to avoid producing.

"We do not use Boxers in our breeding program that could potentially develop ARVC based on the testing currently available," Goselin-Brouillette says. "This means we only breed dogs that test negative for the ARVC gene mutation and have normal results on a color-flow doppler echocardiogram and on a recent 24-hour Holter monitor test. We try to stay informed about the heart health of our Boxers' littermates, sire and dam, and grandsire and granddam."

The same practice prevails when it comes to DM. "Since the DM test became available, we screen all our Boxers. A dog designated at risk for DM is only bred to DM clear dogs. If we have an exceptional Boxer that is a carrier of the *SOD1* gene mutation, we will consider breeding this dog to a DM clear dog, as we can breed away from the mutation in future generations," says Goselin-Brouillette.

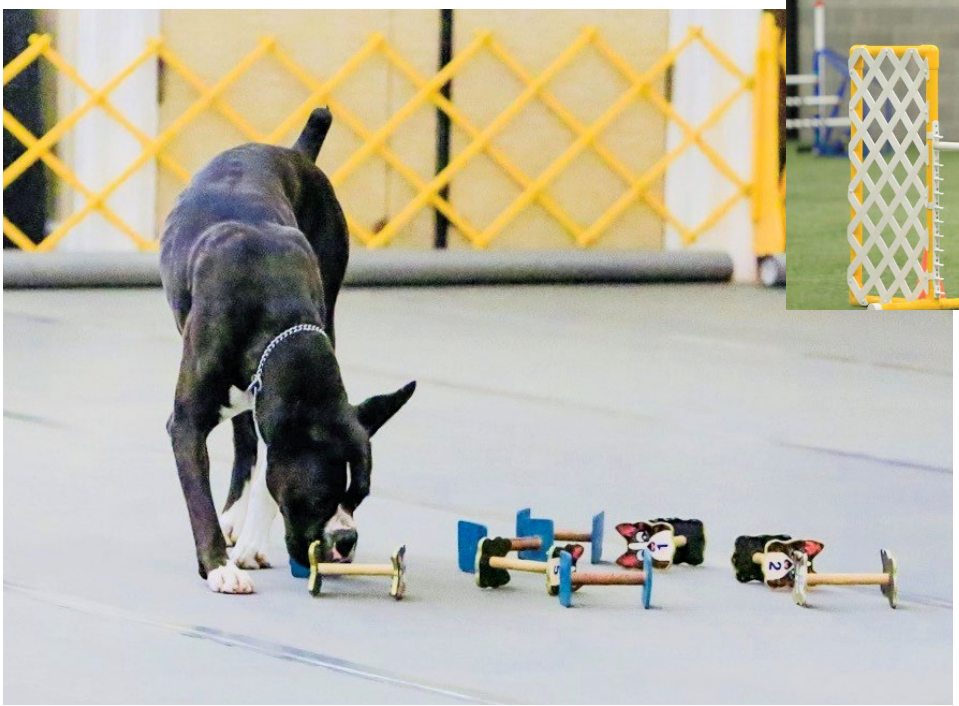
ARVC MUTATION & VARIABLE PENETRANCE

Sadly, arrhythmogenic right ventricular cardiomyopathy is found in Boxers across the U.S. In studies at North Carolina State University, where the ARVC gene mutation was discovered, of nearly 2,000 Boxers tested, 41 percent had one copy of the gene mutation, 6 percent had two copies, and 53 percent were negative.

"Removing nearly half of the Boxers from the breeding population would have a devastating effect on the gene pool," says Kathryn M. Meurs, DVM, PhD, DACVIM (Cardiology), the Randall B. Terry Distinguished Professor of Comparative Medicine and Associate Dean of Research. "Remember, dogs that carry the mutation also carry other important genes that we do not want to lose from this breed."

The lead investigator of the ARVC gene mutation discovery, Dr. Meurs says, "Variable penetrance is poorly understood. We can identify which Boxers have the striatin-deletion gene mutation, but we cannot predict the penetrance. Further, there is likely more than one cause, so even if a dog is genetically negative, it does not mean the dog cannot get ARVC. Multiple genetic and non-genetic factors may contribute to the identical clinical disease."

The average age when clinical signs are observed is 6 years, though age of onset is widely variant as well. "Some Boxers will show signs when they are younger, and some not until they much older," says Dr. Meurs. "By the time clinical signs appear, the disease is typically well-progressed."



"Blayke" (MACH RACH Sunchase's Spin The Bottle UD PCD BN RM2 RAE3 MXS MJS NF CGCA), left, and "Owynne" (CH Sunchase's Signature Black CDX RM RAE AX AXJ CGC TKN) are carriers of the degenerative myelopathy* SOD1 *gene mutation and are clear for the arrhythmogenic right ventricular cardiomyopathy gene mutation. Sunchase Boxer breeders Rhoda Goselin-Brouillette and Tracy Hendrickson regularly use health and genetic testing and stay informed about the health status of dogs in the line. "We do our very best to breed healthy dogs, but nothing is guaranteed," Goselin-Brouillette says.

A BALANCED APPROACH TO BREEDING BOXERS

PHOTO: GAY GLAZBROOK

Taking breeding advice from the "Review of the Current State of Genetic Testing in Dogs" helps put in perspective how to breed healthy dogs with quality traits when inherited diseases crop up in bloodlines.

"Boxer breeders must balance their choices to produce the fewest affected animals while removing the fewest animals from the gene pool to avoid a loss of genetic diversity," says manuscript co-author Anita Oberbauer, PhD, professor at the University of California-Davis. "When selecting mating pairs you should think about the characteristics of individual dogs and the puppies you hope to produce but also consider how you can make choices to improve the breed as a whole."

Published in late 2020, the white paper resource was co-funded by the AKC Canine Health Foundation and the Orthopedic Foundation for Animals. Liza Gershony, DVM, PhD, an AKC Canine Health Foundation Clinician-Scientist Fellow and postdoctoral scholar, co-wrote the manuscript with her advisor, Dr. Oberbauer.

Below are key learnings taken from this paper that apply to genetic testing of Boxers for arrhythmogenic right ventricular cardiomyopathy (ARVC), an autosomal dominant disorder with variable penetrance, and degenerative myelopathy (DM), an autosomal recessive condition with incomplete penetrance.

- Genetic tests for multifactorial diseases, such as ARVC and DM, may indicate a Boxer is at risk for developing a disorder based on having at-risk disease alleles, though the presence of these alleles cannot definitely determine whether or when a dog will develop a disease. Some dogs that test positive at-risk may never develop clinical signs, as these diseases involve multiple genetic and non-genetic factors.
- DM is further complicated genetically due to having incomplete penetrance, meaning a Boxer that tests homozygous for the *SOD1* mutation may be at increased risk but is not guaranteed to develop the disease. An unaffected at-risk homozygous Boxer nonetheless would pass the mutation to his or her offspring.
- To preserve genetic diversity, a quality dog tested as a carrier can be bred to a clear dog free of the mutation. Fifty percent of puppies would be clear and also have the genetic richness of the carrier parent. Clear puppies from this breeding could be used in the next generation.
- Insightful knowledge from genetic testing about the likelihood of a dog to develop a hereditary disease is particularly meaningful for disorders that develop later in life and for which clinical surveillance and/or lifestyle or dietary modifications could improve the dog's quality of life. Proper interpretation of genetic tests is fundamental to avoid misapplication and negatively impact genetic variability.
- Breeders should gradually apply selection based on genetic testing to improve breed health. Most importantly, breeders should not depopulate the breed and cause a genetic bottleneck from loss of genetic diversity. Always be mindful of how your choices impact the population of the Boxer breed as a whole.

Boxer ARVC is a heart muscle disease attributed to a deletion mutation in a gene that produces striatin, a key binding protein of the cardiac desmosome responsible for the heart's electrical functioning and holding cells together. ARVC may manifest as congestive heart failure. Fluid accumulates in the lungs, known as pulmonary edema. Affected Boxers develop a cough, shortness of breath and lethargy.

Boxers with ARVC may develop a run of VPCs, or early contractions of the lower right ventricle of the heart causing disturbed electrical impulses. These impulses direct the heart to beat and to maintain a steady, regular rhythm. A dog having multiple, successive VPCs, or heartbeats without a corresponding pulse, is not able to produce normal, effective contractions. Ultimately, this results in decreased blood flow to the brain and other vital organs. A prolonged run of VPCs can lead to cardiac arrest and sudden death in otherwise healthy adult dogs.

"We found Boxers that were homozygous for the mutation, or had two copies of the gene deletion, developed a more severe form of ARVC based on Holter monitor testing. When these dogs had VPCs, they tended to have more of them," Dr. Meurs explains.

Holter monitor testing can detect VPCs in Boxers suspected of having ARVC. Because the arrhythmia is intermittent, it may not occur during a standard three-minute electrocardiogram test or show on an echocardiogram, an ultrasound of the heart. A Holter monitor test is used to provide information about heart rhythm over a 24-hour period and thus is more accurate at identifying affected dogs than a brief electrocardiogram.

The general breeding recommendation for dogs that are homozygous affected with two dominant disease alleles is not to breed them. "That is unless a particular dog is exceptional, and we need to preserve its positive traits and contributions to the breed," Dr. Meurs says.

"Because these dogs appear to have more significant disease and will certainly pass on the mutation to their offspring, if they are bred they should be crossed with a dog that is negative for the mutation," she says. "Over a few generations, a puppy that is negative for the mutation can be selected as the replacement for the breeding program."

PHOTO: GAY GLAZBROOK

"We can identify which Boxers have the striatin-deletion gene mutation, but we cannot predict the penetrance."

Kathryn M. Meurs, DVM, PhD, DACVIM, the Randall B. Terry Distinguished Professor of Comparative Medicine and Associate Dean of Research, North Carolina State University

GENETIC TESTING FOR ARVC & DM

Testing Boxers at an early age for the adult-onset inherited diseases arrhythmogenic right ventricular cardiomyopathy and degenerative myelopathy allows breeders to recognize dogs with disease alleles. An informed breeding program is important in efforts to reduce the incidence of these diseases in the Boxer breed.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)
The North Carolina State University Veterinary Genetics Lab (VGL) offers a direct DNA test for ARVC. The test identifies dogs as positive homozygous, positive heterozygous and clear for the deletion mutation in a gene that produces striatin, a key binding protein involved with the heart's electrical functioning. The cost to test one dog is $48, and reduced pricing is offered for liters of five or more dogs when submitted together.

Degenerative Myelopathy (DM)
The Orthopedic Foundation for Animals provides testing for the DM genetic test developed by researchers at the University of Missouri who led the discovery of the SOD1 mutation that causes this progressive neurodegenerative disease in Boxers. The $65 test produces results showing clear (normal), carrier and at-risk dogs for DM.

Dogs that are heterozygous affected, or inherit one copy of the ARVC mutation, may develop abnormal heartbeats as well. "However, some dogs with the genetic mutation will never show the disease," Dr. Meurs says. "This suggests that though the mutation affects the heart, it likely requires certain environmental and other genetic factors to develop the severe form of the disease."

As to the value of the ARVC DNA test, Dr. Meurs says, "Mutation testing should be used with health testing. Breeders should use this test as a tool to guide them. For example, they might decide to breed dogs with positive attributes that are heterozygous for the mutation and do not show signs of disease to mutation-negative mates. As noted earlier, we do not recommend breeding Boxers with two copies of the gene mutation unless they are exceptional dogs, and then they should only be bred to mutation-clear mates."

Meanwhile, Dr. Meurs is currently working on a study to identify a second genetic risk allele associated with the development of ARVC in Boxers. The two-year study, which runs until April 2022, is funded by the American Boxer Charitable Foundation, and the AKC Canine Health Foundation administers the grant. The research involves looking at DNA samples from Boxers with confirmed ARVC that are negative for the striatin-deletion mutation.

DM MUTATION & INCOMPLETE PENETRANCE

Boxers are among over 40 breeds and mixed breeds with definitively diagnosed degenerative myelopathy in the breed population. Joan R. Coates, DVM, DACVIM-Neurology, professor of neurology and neurosurgery, and colleague Gary S. Johnson, DVM, PhD, of the University of Missouri, led the discovery of the autosomal recessive mutation in the superoxide dismutase 1 gene (*SOD1*) along with Kerstin Lindblad-Toh, PhD, of the Broad Institute of MIT and Harvard. The team found that the mutated gene in dogs is the same as the one causing some forms of familial amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, in people.

A devastating disease that mimics other neurologic conditions, DM initially impairs a dog's hind limbs. Dragging or scuffing of the legs leads to decreased muscle control and weakness causing frequent falls and difficulty getting up. Within 11 months, dogs are paralyzed, as DM spreads through the nervous system, damaging the spinal cord, brain, nerves, and muscles. Boxers are usually around 9 years of age when signs are recognized, though a definitive diagnosis is not possible until a necropsy and histopathology are performed after death.

When the *SOD1* gene discovery was published in 2009 in the *Proceedings of the National Academy of Sciences*, the investigators stated that the mutation has an age-related incomplete penetrance. The longer an at-risk dog lives, the higher the likelihood of developing signs of DM. However, they noted they did not know the precise risk for dogs having two copies of the mutant *SOD1* variant to developing DM.

"Although the DNA test helps breeders make breeding decisions, it has diagnostic limitations because it is testing for a risk factor and does not provide definitive diagnosis," Dr. Coates says. "Results from genetic testing allow breeders to breed dogs that carry the

PHOTO: GAY GLAZBROOK

SOD1 mutation to clear, healthy dogs to avoid producing affected dogs without reducing genetic diversity.

"We have not yet definitely documented DM by histopathology in Boxers that have tested carrier. DM has been only definitively documented in Boxers testing homozygous or at risk. We continue to collaborate with Dr. Lindblad-Toh to pursue research to seek modifying genes that could influence an individual dog's risks for developing DM. We, too, believe that dogs that carry the mutation also carry other important genes that we do not want to lose from this breed."

The bottom line is to consider each dog and each bloodline individually. "There is no magic formula in breeding Boxers," says Goselin-Brouillette. "We use every tool available to produce healthy dogs. It is heartbreaking not to be able to breed a beloved dog, but that hard decision sometimes needs to be made. Even worse is the risk of producing a dog with a disease like ARVC or DM. As breeders, we should be as honest as we can in making breeding decisions." ■

Purina thanks Dr. Joyce Campbell, chair of the American Boxer Club Health and Research Committee and a trustee of the American Boxer Charitable Foundation, for helping us to identify topics for the *Boxer Update*.

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