PURINA PRO PLAN

BOXER Update

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ARVC2 GENE VARIANT IDENTIFIED

Another Piece to Aid Understanding of the Boxer Heart Disease

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DISCOVERY OF SECOND ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY GENE VARIANT IN BOXERS

PROVIDES ANOTHER TESTING TOOL

PHOTO: ALEXANDRIA GAV

"Wyatt" was diagnosed with ARVC at 3 years of age, earlier than the average age of 6 years. Despite having the heart disease, he went on to earn titles in obedience, rally and coursing ability. When he passed away in August 2021 at nearly 9 years of age, his owner said she was blessed to have had an extra five and half years with him after the diagnosis.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a Boxer heart disease with a lot of loopholes. Although a dog may inherit a copy of the ARVC1 or ARVC2 deletion mutations, some Boxers never develop clinical signs. Others have mild, medically manageable cases. Then, there are those Boxers that have neither mutation, yet they develop the potentially fatal disease.

ARVC occurs in adult dogs on average at 6 years of age, often after they have been bred. The inherited disease affects the heart's electrical system and too often results in sudden death or congestive heart failure.

The latest discovery in 2021 of a second gene variant (ARVC2), believed to be an autosomal dominant mutation, in a regulatory gene involved in important cardiac proteins comes with a disclaimer: *Incomplete Penetrance*. Translated, this means that not all Boxers testing positive for ARVC2 will develop the disease.

A sense of déjà vu prevails. In 2008 when the first gene variant (ARVC1) was identified in a gene producing striatin, a key binding protein that holds cells together, it also was described as having incomplete penetrance. Some dogs that inherit a copy of the autosomal dominant ARVC1 deletion mutation die suddenly following a run of ventricular premature complexes (VPCs), yet others succumb over time from congestive heart disease. As described

earlier, some dogs do not show clinical signs or are mildly affected.

"Incomplete penetrance is a poorly understood concept that is very common in human genetics as well. The human form of ARVC is also complicated by incomplete penetrance," says Kathryn M. Meurs, DVM, PhD, DACVIM (Cardiology), the Randall B. Terry Distinguished Professor of Comparative Medicine and Associate Dean of Research at North Carolina State University. "In Boxers, we can identify which ones have the ARVC1 and ARVC2 gene mutations, but we cannot predict the penetrance. This suggests that multiple genetic and nongenetic factors may contribute to whether a dog develops this disease."

In humans, the rare, familial heart disease has been linked to 141 mutations in eight genes. As with dogs, fatty, fibrous tissue replaces normal heart tissue and interrupts the heart's normal electrical functioning. Occurring in young, apparently healthy individuals, ARVC may cause ventricular tachycardia and sudden cardiac death.

"The functionality of the genes we have identified in Boxers is very similar to what is currently known about the disease in humans," Dr. Meurs says. "We have known for several years not all Boxers that develop ARVC have the striatin gene mutation. It is similar to humans where there are multiple different genes that can cause ARVC."

The quandary of another possible genetic cause is what prompted the American Boxer Charitable Foundation (ABCF) to fund the latest study that produced the ARVC2 gene variant, a single-nucleotide polymorphism affecting the transcription factor of a regulatory gene. The AKC Canine Health Foundation administered the grant.

Longtime ABCF president Dr. William Truesdale says, "We encouraged Dr. Meurs to work to see if there was another cause of ARVC. She is the pioneer of this disease. Our collaboration led to her finding the first ARVC mutation after seven years of research. At the time, we were elated, though we were seeing some dogs that were negative for ARVC1 that had clinical signs and were dying."

"Breeders should use mutation testing along with health testing," Dr. Meurs advises. "These tests are tools to guide breeding decisions rather than to be used as an absolute recommendation for all dogs. Dogs that carry these mutations also carry important good genes that we do not want to lose from the breed.

"A Boxer with positive attributes that is heterozygous for one or both mutations and does not show signs of disease could be bred to a mutation-negative mate. A dog from this breeding that tests negative for ARVC1 and ARVC2 and does not have clinical signs could be used to produce the next generation."

Veterinarian Robert C. Hallock of Cheshire, Connecticut, who breeds Boxers with his wife, Grace, under the Pearlisle prefix, says, "ARVC

CHIC HEART TESTS FOR BOXERS

Among the recommended tests for Boxers to be eligible for certification by the Canine Health Information Center (CHIC) are two tests to help identify dogs at risk for arrhythmogenic right ventricular cardiomyopathy (ARVC):

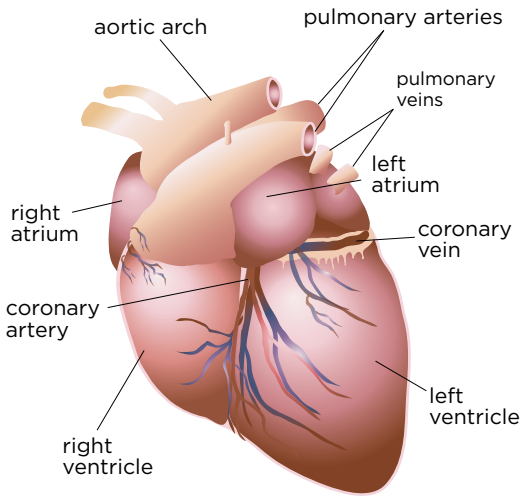
- An advanced cardiac examination by a board-certified veterinary cardiologist that includes a Holter monitor test
- ARVC DNA test

The Orthopedic Foundation for Animals, working with the American Boxer Club, recommends these screening tests, along with a DNA test for degenerative myelopathy and testing for hip dysplasia and autoimmune thyroiditis, for all breeding stock. Dogs meeting these basic health screening requirements will be issued CHIC numbers. Note that for CHIC certification, a dog's testing results do not need to be normal but must be made public so that responsible breeders can make informed breeding decisions. In addition to these health requirements, a dog must be permanently identified via a microchip or tattoo to qualify for a CHIC number.

GENETIC TESTING FOR ARVC IN BOXERS

Genetic testing for the ARVC1 and ARVC2 gene variants is available via the Veterinary Cardiac Genetics Laboratory at North Caroline State University. The cost for a combined test for ARVC1 and ARVC2 is $70, and each test separately is $48.

UNDERSTANDING HEART MALFUNCTIONING IN BOXERS WITH ARVC



In a normal heart, the left atrium contracts to push blood into the lower right ventricle, and then the ventricle contracts to push the blood out to the body. Boxers with ARVC may develop ventricular premature complexes (VPCs), in which the ventricle contracts earlier than it should and thus cannot produce a normal, effective contraction. If multiple, successive VPCs occur, this results in stopping blood flow to the brain and other organs, potentially causing sudden cardiac death.

Over time, the heart of Boxers with ARVC dilates to compensate for the weakened heart muscle. As the thinned heart walls continue to weaken, the diseased heart muscle fails to generate enough force to pump the blood. As pressure rises in the left atrium, it leads to fluid accumulation in the lungs, known as pulmonary edema. Congestive heart failure follows.

is frustrating because you usually can't diagnose it when dogs are young. You may have a good dog that goes through its show career and you want to add this dog to your breeding program only to learn it is clinically affected by ARVC."

This was the case with the Hallocks' Top 20 Boxer bitch "Meghan" (GCHB Pearlisle's Trial By Fire CGC). "Meghan was negative for the ARVC1 mutation. We did not yet have the test for ARVC2," Dr. Hallock says. "She was clinically affected and died in 2017 at 8 ½ years of age."

Sometimes a breeder has to start over when ARVC runs through a bloodline. "This disease was devastating to my breeding program," says Trish Olinghouse of Lando Boxers in Neosho, Missouri. "I basically had to start over when my male 'Wyatt'

PHOTO: ALEXANDRIA GAV

Trish Olinghouse is shown with Wyatt, left, and "Nike," whom she bought to start over her bloodline after Wyatt, from her fourth generation, was diagnosed with ARVC.

(BISS CH Lando's I'm Your Huckleberry RN CGC CA BN) was diagnosed with ARVC."

A self-described stickler about Holter monitor testing, Olinghouse also screens her Boxers for the genetic mutations. "Since Wyatt's parents tested negative for ARVC1, the only test available at the time, I thought the odds of him developing ARVC were slim," she says. "I was getting ready to Special Wyatt when he had an abnormal Holter monitor test at 3 years of age.

"He was healthy and fit, asymptomatic. After the abnormal Holter monitor test, a veterinary cardiologist diagnosed ventricular tachycardia, a fast, abnormal heart rate that can be life-threatening. Wyatt was sicker than we thought. I brought him home on antiarrhythmic medications, mexiletine and sotalol, which he took for the rest of his life."

The sweet-tempered Wyatt loved people and going to dog shows and training. "Wyatt didn't know he was sick," Olinghouse says. "It was important to me to allow Wyatt to remain active and happy. After the diagnosis, he went on to earn the Coursing Ability, Rally Novice and Beginner Novice titles."

After researching bloodlines the year after Wyatt was diagnosed with ARVC, Olinghouse bought "Nike," a bitch with European and North American relatives, who has tested negative on both ARVC tests. OHBIS/BISS GCH Rocket N Lando VaVa Voom BN RN CAA CGC TKN RATI would carry Lando Boxers into the future — hopefully one free of dogs affected by the crushing heart disease.

UNDERSTANDING THE DYNAMICS OF ARVC

Although arrhythmogenic right ventricular cardiomyopathy occurs in Boxers on average at 6 years of age, some dogs — like Wyatt — show clinical signs when they are younger and some when they are older. "By the time clinical signs appear, the disease is typically well-progressed," Dr. Meurs explains.

Boxers may experience a run of ventricular premature complexes, or early contractions of the lower right ventricle of the heart. Fatty, fibrous tissue that develops in the heart muscles causes these disturbed electrical impulses rather than a normal, steady, regular rhythm.

A dog having multiple, successive VPCs, or heartbeats without a corresponding pulse, is not able to produce normal, effective contractions, which results in decreased blood flow to the brain and other vital organs. A prolonged run of VPCs — the ventricular tachycardia that Wyatt experienced — can lead to cardiac arrest and sudden death in otherwise healthy dogs.

HOLTER MONITOR TESTING FOR BOXER ARVC

PHOTO COURTESY OF WASHINGTON STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE

A Holter monitor that records a dog's heart rhythm continuously over 24 hours has become the gold standard test to identify dogs potentially at risk for developing the heart disease arrhythmogenic right ventricular cardiomyopathy (ARVC). A lifesaver, the Holter monitor allows treatment to begin before a dog shows clinical signs.

"Kate Meurs (Dr. Kathryn Meurs of North Carolina State University) has virtually singlehandedly created a 'Holter monitor culture' in North America that has greatly reduced the incidence of early death in Boxers to ARVC," says William Truesdale, DVM, president of the American Boxer Charitable Foundation.

The size of a credit card worn in a vest with two leads to a dog's chest, the Holter monitor effectively picks up ventricular premature complexes (VPCs), which cause arrythmia, the erratic heartbeats that can result in cardiac arrest and sudden death. In contrast, an electrocardiogram captures heart electrical activity for a brief period while a dog is at rest, thus it may miss the intermittent VPCs common in Boxers with ARVC.

An abnormal Holter monitor test predicts dogs that may later experience cardiac arrest or develop congestive heart failure. Importantly, it enables diagnosis so that veterinary cardiologists can begin treatment with beta blockers and antiarrhythmic medications to help prevent severe arrhythmia or congestive heart failure. It is recommended that Boxers receive annual Holter monitor tests starting at age 3.

TIMELINE OF ARVC DISCOVERIES IN BOXERS

EARLY
1970s
Boxers, even young, healthy adult dogs, begin dying suddenly. Some had fainting episodes during the months preceding their sudden death, though others had no signs prior to dying. Some of the breed's most influential producers are among those that "just dropped dead."

EARLY
1980s
Now called Boxer cardiomyopathy, the condition is believed to be hereditary. Boxers often have already been bred when clinical signs develop.

1997
While at The Ohio State University, Dr. Kathryn M. Meurs determines that Boxer cardiomyopathy is inherited as an autosomal dominant trait based on DNA analysis from families of Boxers. The research, supported by ABCH and the AKC Canine Health Foundation, was published in 1999 in the *Journal of Veterinary Internal Medicine*.

1997-99
Holter monitor testing of six large families of Boxers at The Ohio State University finds that the disorder is primarily a familial heart muscle disease associated with substantial cardiovascular morbidity and risk of sudden death. Boxers are deemed a new animal model for the human disease. The research was published in 2004 in the journal *Circulation*.

1995
The American Boxer Charitable Foundation (ABCF) conducts a breed health survey that indicates heart disease is the top health concern of Boxer breeders.

2004
Dr. Meurs moves to Washington State University and continues this research. She renames the fatal heart disease, arrhythmogenic right ventricular cardiomyopathy (ARVC), to more precisely describe the condition. She receives a three-year research grant from the AKC Canine Health Foundation to study risk factors associated with ARVC, with a goal of developing screening methods to identify asymptomatic affected dogs before they are bred.

ARVC in Boxers is characterized by fatty or fibro-fatty replacement of the right and sometimes left ventricular myocardium and by ventricular tachycardia. Although inherited as an autosomal dominant trait, incomplete penetrance could be involved due to the variance in presentation. The findings were published in *Veterinary Clinics of North America: Small-Animal Practice*.

2008
Dr. Meurs is the lead investigator of research that identifies the ARVC1 deletion mutation in the striatin gene responsible for the heart's electrical functioning and holding cells together. The findings that three of 33 dogs studied lacked the striatin mutation suggests there is at least one other cause of the disease in Boxers. This study was published in *Human Genetics* in 2010 and the *Journal of Veterinary Internal Medicine* in 2013.

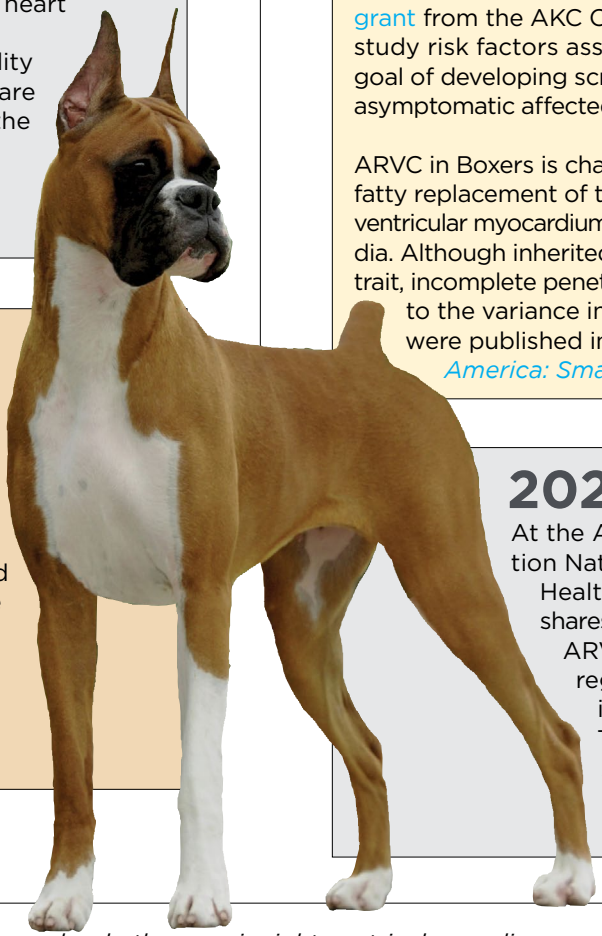


PHOTO: GAY GLAZBROOK

2021
At the AKC Canine Health Foundation National Parent Club Canine Health Conference, Dr. Meurs shares that a second gene variant, ARVC2, has been found in a regulatory gene involved in important cardiac proteins. The research was funded by ABCF and administered by the AKC Canine Health Foundation.

Editor's Note: The Boxer Update *has covered arrhythmogenic right ventricular cardiomyopathy more than any other topic. Counting the 2022 issue, we have published six stories starting with our first issue in 2002 that have reported on the disease and research progress.*

Meanwhile, Boxers can develop congestive heart failure when fluid accumulates in the lungs, a condition known as pulmonary edema. Affected Boxers develop a cough, shortness of breath and lethargy.

The ARVC1 and ARVC2 gene variants help to explain why the heart malfunctions in clinically affected dogs. ARVC1 is attributed to the deletion mutation in the striatin gene that produces a key binding protein of the cardiac desmosome responsible for the heart's electrical functioning and holding cells together. Dr. Meurs' initial ARVC1 study found that 53 percent of Boxers were negative, 41 percent were positive heterozygous, and 6 percent were homozygous.

In discovering ARVC2, Dr. Meurs and her team at North Carolina State University used whole-genome sequencing on DNA from Boxers with confirmed ARVC that were negative for ARVC1. Their findings showed that 75 percent of the ARVC2 heterozygous-positive Boxers showed clinical signs and 25 percent did not.

"About one-quarter of dogs have both mutations. We are working to determine if dogs with both mutations have more severe disease," Dr. Meurs says. "Our initial data suggests that they do have more severe disease, as it appears that they have a higher number of arrhythmias and abnormal heart muscle functions. There are still a few affected dogs that have neither mutation."

ANOTHER TOOL FOR TESTING BOXERS

Reflecting on the progress that has been made over the past 20 years, Dr. Meurs says, "The dedication and commitment of the American Boxer Charitable Foundation to improve the health of the Boxer breed is beyond what I have seen in any other breed organization."

Whereas 50 percent of Boxers were clinically affected by ARVC 20 years ago, Dr. Meurs is not certain of the exact percentage today but notes that the number of homozygous positive dogs has reduced by about 60 percent. "We only see about 3 to 4 percent of all dogs to be positive homozygous," she says.

Breeder Trish Olinghouse laments on the ongoing challenges. Her Boxer bitch, Nike, whom she bought to start over her Lando Boxer bloodline, produced an outstanding male, "Ford" (CH Lando N Rocket's Pickup Man @ Krisdan CA BCAT). However, Ford turned out to be a carrier for ARVC2, though he is clear for ARVC1. "We can't take all carriers out of the breeding program," she says. "I plan to selectively breed him to bitches that are clear and will continue to Holter monitor test him every year."

Dr. Meurs advocates that practice. "Each dog and each family line should be considered individually," she says. "The removal of a significant number of dogs from the breeding population could be very bad for the Boxer breed. The field of canine genetics is very new, and the field of cardias genetics is very complex. We are still learning how to use these tools."

Dr. Truesdale, Dr. Hallock and Olinghouse agree that the DNA tests are important pieces of the puzzle. They also are open to the possibility that other gene variants and even nongenetic factors may contribute to this disease in Boxers.

"It's heartbreaking that this disease plagues the Boxer breed," Olinghouse says. "As a community, we cannot better the breed if we don't get our health right." ■

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 this topic for the *Boxer Update*.

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