

The Portuguese Water Dog Foundation, Inc.®

We are dedicated to funding canine medical research focused on issues that affect the health and well-being of Portuguese Water Dogs everywhere.

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GETTING TO THE BOTTOM OF ADDISON'S DISEASE

By Steven G. Friedenberg, DVM, PhD, DACVECC

As many of you know, Addison's disease is an autoimmune disorder that affects many PWDs. The disease targets the adrenal glands, which are small organs that are located right in front of the kidneys in dogs. The adrenal glands make several hormones that are extremely important in the body, namely cortisol and aldosterone. In Addison's disease, the immune system inadvertently attacks the adrenal glands, and affected dogs can no longer make one or both key hormones. This leads to a host of clinical signs, ranging from a waxing and waning appetite to cardiovascular collapse and sudden death.

Portuguese Water Dogs have a very high risk of developing Addison's disease relative to other dogs. According to one study, PWDs have nearly a 30-fold increased risk of developing Addison's! Because of this, it's important to be aware of the disease and talk to your veterinarian if you think your dog might be affected.

Given that the disease is so prevalent in PWDs, there is highly likely a genetic component to it in PWDs. Unlike some genetic diseases, however, Addison's disease is likely to be what geneticists refer to as a "complex" trait. This means that there is *not* likely to be a single gene that causes the disease, but rather multiple genes. On top of that, genes are *not* likely to explain the whole story: There is likely some type of

environmental exposure as well that is required to trigger the disease onset. All of these factors make understanding the genetics of Addison's disease tricky, but we are working hard to unlock the riddle.

The Portuguese Water Dog Foundation is currently funding a large study at the University of Minnesota and Clemson University designed to try to understand what are the genetic mutations that predispose PWDs to developing Addison's disease (z.umn.edu/pwdaddisons). The study is being led by Drs. Steven Friedenberg and Leigh Anne Clark, and is actively recruiting participants.

If you have a PWD with Addison's or if you have a healthy PWD who is 9 years of age or older and would like them to participate, please email pwdstudy@umn.edu for more information. The study coordinators will start by asking for a pedigree from your dog so that they can evaluate how related he or she is relative to the rest of the study population. If your dog qualifies, Dr. Friedenberg will work with your veterinarian to collect a small, one-time blood sample from your dog and send it to his lab in Minnesota. The study will pay for all expenses associated with the blood draw and shipping the sample to the lab. The more participants the study can enroll, the greater the chances of finding a test that can one day help make PWDs less likely to develop this disease than they are today.

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PROGRESS REPORTS

Pattern of Thyroid Function Tests during Recovery from Acute Nonthyroidal Illness*Timothy Bolton, DVM, Virginia-Maryland Regional College of Veterinary Medicine*

Hypothyroidism is the most common endocrine disease in dogs. A diagnosis of hypothyroidism relies on finding both appropriate clinical signs and low thyroid hormone levels. Unfortunately, other illnesses can suppress thyroid hormone levels and result in a misdiagnosis. This phenomenon of low thyroid hormone levels caused by a disease not involving the thyroid gland is known as nonthyroidal illness or euthyroid sick syndrome. It is important to distinguish between nonthyroidal illness and hypothyroidism as the treatment for each is different. Historically, the recommendation for a dog with nonthyroidal illness has been to resolve the underlying disease, followed by a recheck of thyroid hormone levels thereafter. However, the duration of time after resolution of the nonthyroidal illness necessary to perform accurate thyroid hormone level testing is unknown. This study will provide information about thyroid hormone levels during the course of nonthyroidal illness, and also establish the approximate duration of time for recovery of thyroid hormone levels to normal following illness resolution.

To date, 93 cases have been screened for possible study enrollment. Of these, 23 have fulfilled all of the enrollment criteria (92% of total cases needed) and are still in the study, 7 were enrolled but ultimately excluded (either determined to have a chronic disease, did not follow-up, or determined to have overt or subclinical hypothyroidism), 8 were not enrolled despite having a low total T4 (owner did not allow for study enrollment or found to have a chronic disease during diagnostic work-up), and 55 had a normal total T4 (failed to fulfill this inclusion criteria).

Based on the case enrollment thus far, 19/21 dogs had normalization of total T4 by the 2-week recheck. Thus, in dogs where acute nonthyroidal illness is diagnosed, rechecking the total T4 two weeks after nonthyroidal illness resolution will result in an accurate assessment of thyroid hormone status for most patients. For those not normal at 2-weeks, a recheck again 2-weeks later (at the 4-week mark) would be most appropriate, as all dogs had a normal total T4 by 4 weeks.

As expected, 100% of dogs at that time of nonthyroidal illness diagnosed had a low total T4 and ~90% had a low total T3. These values returned to normal as the disease process resolved. Free T4 (low) and TSH (high) was normal in all but 8 cases. The TSH, in most cases, appeared to significantly increase at the 2- and/or 4-week rechecks, albeit still within the reference range. Interestingly, in three cases, the TSH increases above the reference range after normalization of all other thyroid hormones (total T4, free T4, total T3) prior to returning to normal.

Enunciating differences between the pattern of thyroid hormone recovery in dogs and humans with naturally occurring disease will be postponed until all cases are enrolled and analyzed.

Identification of Bartonella henselae In Vivo Induced Antigens for Development of a Reliable Serodiagnostic Assay for Canine Bartonellosis*By Edward Breitschwerdt, DVM, North Carolina State University*

We are on track to accomplish all of our aims for this study. Due to COVID-19 pandemic and backorders of supplies, we had a short delay in starting the study. We have now received all the lab supplies. Screening of Bh SA2 expression genomic library by colony immunoblotting (Aim1) require additional time and research effort to identify potential diagnostic antigens for canine bartonellosis. Once we identify potential diagnostic markers, we will evaluate utility of these proteins and develop a reliable ELISA for serodiagnosis of Bartonella infection in dogs.

Bartonella, a genus of gram-negative bacteria, are associated with a wide spectrum of life-threatening diseases in animals and humans. More than 40 Bartonella species have been reported to infect mammalian reservoir hosts, and infection often leads to chronic bacteremia. At least ten Bartonella species have been implicated in association with serious diseases in dogs, including endocarditis, hemangiosarcoma, myocarditis, peliosis hepatis, polyarthritis and vasculitis.

PROGRESS REPORTS CONTINUES

Canine Bartonellosis, continued

Despite biomedical advances and ongoing research in the field of canine bartonellosis, currently available PCR, culture, and serological based assays lack sensitivity for diagnosis of bartonellosis. Dogs throughout the United States and much of the world are exposed to *Bartonella* species. From a public health perspective there is an increased risk of direct and vector-borne transmission of *Bartonella* species from animals to humans. These factors justify the need for the ongoing development of a reliable serodiagnostic modality and ultimately an effective vaccine for prevention of bartonellosis in dogs. We will employ In-Vivo Induced Antigen Technology (IVIAT) to identify *Bartonella* in-vivo induced antigens, which will allow us to evaluate their potential as diagnostic markers for canine bartonellosis. This proposed study will result in development of a novel and sensitive ELISA assay for diagnosing *Bartonella* infection in dogs and will provide insights into the development of effective vaccine candidates for preventing *Bartonella* infection.

Scientific and Clinical Assessment of Fecal Microbiota Transplant in Obese Dogs: SLIM Study

Jenessa Winston, DVM, PhD, The Ohio State University

To date, we have screened (comprehensive physical examinations, bloodwork, and fecal analysis) 51 dogs for the SLIM study. Of these, 31 have met the inclusion criteria and were enrolled. Of these enrolled dogs, 23 dogs have completed the 24-week clinical trial and 2 dogs are currently enrolled (see Figure 1). Unfortunately, 6 dogs were removed from the study early with the most common reason related to administration of antimicrobials needed for underlying health conditions including dog bite wounds, urinary tract infections, and surgical procedures. Based on recalculating our power analysis for sample size estimation, our current sample size (n=25) is sufficient to find a significant difference in weight loss between the treatment groups. Therefore, we are not planning to recruit any additional dogs for the SLIM study. All currently enrolled dogs will finish the study in October 2022 and then we can enter the analysis phase of this clinical trial.

The most rewarding part of this clinical trial is that all dogs are achieving their weight loss goals, despite their treatment group. Our patients' owners are thrilled with how their pets are progressing in their weight loss goals and are greatly appreciative for the improvement in their pets' quality of life since completing the SLIM study. For example, over the 24-week SLIM study, Pax's achieved a total weight loss of 23% of his body weight (see Figure 2). His body condition score (BCS) improved from 8/9 to 5/9 during the SLIM study. His owners' report a marked improvement in his energy and active levels since completing the SLIM study. Our institution continues posting social media stories about our patients' weight loss journeys.

Bladder Carcinogen Exposures in Pet Dogs

Dr. Lauren Trepanier DVM, PhD, University of Wisconsin, Madison

Bladder cancer is an aggressive cancer that affects ~ 20,000 dogs per year, and often leads to euthanasia. Certain breeds have a higher incidence of bladder cancer but genetic studies even in the highest risk breeds have been inconclusive and still indicate influence from environmental exposures.

The investigators propose that specific household environmental chemical exposures contribute to the risk of bladder cancer in dogs. In this study, they will measure urinary concentrations of five different chemicals that are known or suspected to be bladder carcinogens, in dogs with bladder cancer compared to unaffected dogs. The investigators will determine whether the presence of certain chemicals is associated with household exposures, based on owner questionnaires and household proximity to industrial sites. Finally, they will determine whether urinary chemical concentrations are linked to early DNA damage in the urinary cells of healthy dogs that do not have bladder cancer. The overall goal of this study is to provide veterinarians and dog owners with evidence-based bladder cancer prevention strategies.

Bladder cancer case recruitment has been steady through our alliance with Antech, which is attaching our study flyer to all BRAF urine tests that are positive for bladder cancer. We are now enrolling 2-3 dogs with bladder cancer per month. We

also have a list of possible matched controls through our expanded outreach through Facebook. We have recorded questionnaire data encoded for all dog kits that have been returned to us.

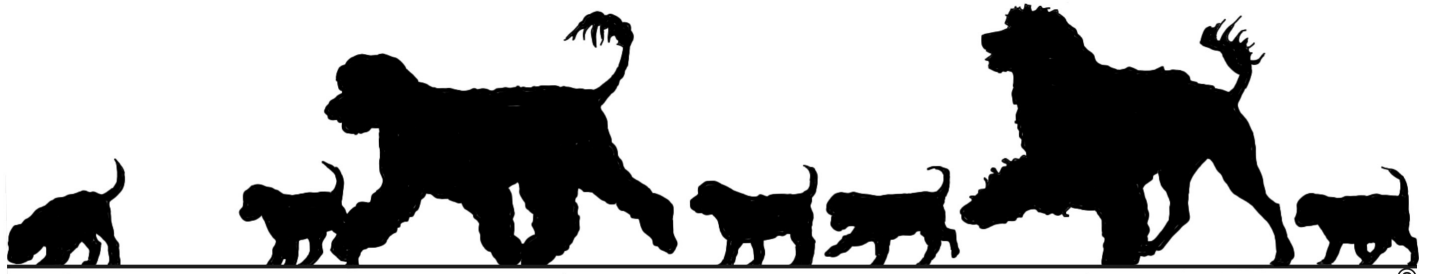
We also obtained additional internal funding to add analyses of arsenic in tap water and household dust, acrolein in household air, and metabolites of acrolein and arsenic from owner urine, in a subset of dog households. Our hypothesis is that these household and owner chemical exposures will be higher in TCC case homes compared to TCC control homes. These data will be included in the final publication.

Transcriptional Profiling of Canine Soft Tissue Sarcoma

By Andrew Miller, DVM, Cornell University

Soft tissue sarcomas account for 10-15% of all skin and subcutaneous cancers in dogs. Traditionally, biopsy and subsequent histology have been the primary means of diagnosing these cancers. The histology is assigned to one of three grades ranging from low (grade I), intermediate (grade II), and high (grade III). Histologic grade is currently the key criterion for guiding treatment and determining patient outcome. However, in human medicine and pathology, soft tissue sarcomas are diagnosed with a hybrid approach that involves both histologic features and genetic analysis of the tumor sample. This genetic analysis guides further treatment, aids in developing accurate follow-up information, and has been shown to have a positive effect on patient outcome and survival. Despite how common soft tissue sarcomas are in the dog, current veterinary care still relies solely on the histologic grade, which is subjective at best, and does not incorporate genetic data into the diagnostic plan.

In the dog, STSs arise frequently in the dermis/subcutis and represent up to 15% of the neoplasms in this location. Our primary aim of this grant was to collect cases of canine STS for histologic and gene expression analysis. During the first two years of the grant, we have had an excellent recovery rate for our cases with very few being dropped due to quality control issues. Now that we have started the 3rd year, we have chosen the last 100 cases and they are currently being processed.



The Portuguese Water Dog Foundation, Inc.®

**The Portuguese Water Dog Foundation, Inc.
500 Westover Drive #30318
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The Portuguese Water Dog Foundation, Inc. needs your help and support to fund research to improve the quality of life and health of our Portuguese Water Dogs. Your donation, in any amount, would be greatly appreciated. In addition to personal donations, a donation may be made in memory or honor of a friend or loved one, whether human or canine. Donors' names will be kept anonymous upon request.

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Ginnie & Bob Santoli in memory of all of our Portuguese Water Dogs

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Roberta Capuano & Thomas Heffernan in thank you to Jane Freeman for trusting them with Freestyle babies and guiding them

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Anonymous

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Dan Dietrich

Dawn Skelly in memory of Chloe who left us too soon

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Linda Hunt, Kalista, in memory Kalista's Kiawah Katie, who is loved and missed by Barb and Bob Fry

Linda Hunt, Kalista, in congratulations to "Sprite" Kalista's Serendipity NA NAJ RN SCM SEM SIM SWE CGC TKI for her SIM

Linda Hunt, Kalista, in congratulations to "Hydro" CH Kalista To Splash-N-Dash MX MXJ T2B TD RN BN SWM SCME CGC TKI WWDX GROM for his SCME

Patricia Belliveau, in honor of Brenda Brown

Linda Hunt, Kalista, in memory of Darleen Cassidy who is loved and missed by Jim and Xena

Linda Hunt, Kalista, in memory "Whoopsie" CH Bayswater's What About Whoopsie CDX RN OA NAJ OAP OJP CWDX who is loved and missed by Mary-Kay and Jerry Schroeder

Karen Pratt in memory of "Zada" Avida's Malazada Doce who was loved, bred, and owned by Chris & Elaine Rasmussen

Linda Hunt, Kalista, in memory of Mallie, loved and missed by Susan & Gary Wahman and Oso

Linda Hunt, Kalista, in honor of "Dario" Kalista's Double Dare TKN for his TKN

Southern California Portuguese Water Dog Club in memory of Questar's Prevailing Winds, JWC, NA, NAJ, NF, OF, RN, CL3-F, CL3-H, CL3-R, owned and loved by Logan Ott and Marilyn Rimmer

Linda Hunt, Kalista, in congratulations to "Bender" CH Kalista's Breaking Bad RN TKN for his CH

John Bracken in memory of Tucker, loved by the Benedetto family

Linda Hunt, Kalista, in congratulations to "Lyra" Kalista's Vega A Lyrae RI SWE SENE SIM TKI AWD for her SIM

Linda Hunt, Kalista, in congratulations to "Hydro," CH Kalista To Splash-n-Dash MX MXJ T2B TD RN BN TKI SWM SCME SWD CGC WWDX GROM MAC-12 for his SWD

David Whittington in honor of Addison's research

Linda Hunt, Kalista, in congratulations to Kalista's Diving Duke TKN for his TKN

Linda Hunt, Kalista, in congratulations to "Trip" Kalista's Asked 'N Answered CD PCD BN RN for his CD

Sandra Novicki in memory of Sailor

John & Diane Burke

Southern California Portuguese Water Dog Club in memory of "Quatro" GCHG CH Roseknoll Sunny Benjamin loved by Ellen Leyda

Linda K. Hunt, Kalista, in memory of Bear, owned and loved by the Howe family

Linda Hunt, Kalista, in memory of Mosey, loved K. and missed by Wendy Beaton-Lanz and family

Linda K. Hunt, Kalista, in memory of "Digger," loved and missed by the Seiler family

Linda K. Hunt, Kalista, in memory of "Proby" who was loved and is missed by Scott Semo, Delores Flynn and David Larson

Linda K. Hunt, Kalista, in honor of "Tally" GCHB CH Kalista's What A Catch RA OA OAJ NF TKI WWD for her RA

Linda K. Hunt, Kalista, in honor of Ch Kalista's Breaking Bad BN RN TKN for his BN

Linda K. Hunt, Kalista, in honor of "Titan" CH MACH3 Sun Joy's Back In Time At Kalista RI MXC MJS2 PJD MFB T2B2 for his MACH3

Linda K. Hunt, Kalista, in memory of Kalista's Little Dob'll Do Ya RA OA OAJ NJP NF AWD "Fiddle" loved and missed by Moe Flaherty

Anne Herberholz in memory of "Howling Holly" loved and missed by the Herrmann Family

Linda K. Hunt, Kalista, in congratulations to "Yikes" Kalista's Already AWOL OA OAJ XF WWD for her WWD

Linda K. Hunt, Kalista, in congratulations to "Finnegan" Kalista's Eagle Eyrie JWD for his JWD

Linda K. Hunt, Kalista, in congratulations to "Tally" GCHB CH Kalista's What A Catch RA OA OAJ NF TKI WWDX for her WWDX

Dan Dietrich

Linda K. Hunt, Kalista, in congratulations to "Reggie" CH Kalista's World On A String for his CH

Deck Hand up to \$50

Deana Allen

Linda K. Hunt, Kalista, in congratulations to "Lyra" Kalista's Vega A Lyrae RI SWE SENE SIM SEM TKI AWD for her SEM

Linda K. Hunt, Kalista, in congratulations to "Titan" CH MACH3 Sun Joy's Back In Time At Kalista RI MXC PAD MJS2 PJD MFB T2B2 for his MACH 3

Linda K. Hunt, Kalista, in congratulations to "Yikes" Kalista's Already AWOL AX AXJ XF BCAT for her BCAT

Linda K. Hunt, Kalista, in congratulations to "Roma" Kalista Bonny Billow Diver FDC BCAT CGCA CGCU TKI for her BCAT

Larry Mitchum

Ylisa Kunze in memory of Sharon Siskind, may her memory be for a blessing

Linda K. Hunt in congratulations to "Tally" GCHB CH Kalista's what A Catch CD RA OA OAJ NF TKI WWDX for her CD

Linda K. Hunt, Kalista, in congratulations to "Oscar" Kalista's Z Golden Boy TKN for his TKN

Carol Cooke in memory of Randy Latham at Christmas

Ryan Heemann

Southern California Portuguese Water Dog Club in memory of "Rosie" Seashell's Steppin' Out In Style BN RE NAJ SWA SBX THDX CGCA TKN NW1, loved by Pam Francis

Southern California Portuguese Water Dog Club in memory of "BoyBoy" GCH Seashell's Steppin' Out With My Baby BN RA THDX CGCA TKN, loved by Pam Francis

Southern California Portuguese Water Dog Club in memory of "Kiara," CH Toraq's Red Skies At Night TKN, loved by Rebecca Morin

Southern California Portuguese Water Dog Club in memory of "Dash," Questar's Black Diamond Bay NJP loved by Martin and Leslie Katz

Anonymous

Martha Barnwell

G.D. Jamerson

Richard Kesin

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Brent Lee

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Marianne Killackey

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Nancy Perry

Special thanks to... Nutmeg PWDC

Every year, the Nutmeg Portuguese Water Dog Club donates proceeds from calendar sales to The Foundation.

Over the years, Nutmeg has donated more than \$50,000 to help fund health for our dogs!

The yearly calendar features 30 action-packed photos of Portuguese Water Dogs from all over the United States and other countries around the world - Austria, Canada, Czech Republic, Denmark, the Netherlands, Norway and Poland.

Submit your photos of your dogs and buy a calendar to help support canine health research.

IN THE SHOES OF... ELIZABETH A. GREIF, MS, PHD CANDIDATE OF GENETICS

My name is Lizzie Greif, and I am the graduate student working with Drs. Steven Friedenberg and Leigh Anne Clark on the genetic investigation of Addison's disease in Portuguese Water Dogs (PWD).

I have been interested in Addison's disease since the start of my research quest. I studied Addison's disease for my first project as an undergraduate student, where I compared different alterations of specific immune system genes (part of the major histocompatibility complex) to see if any were linked to Addison's disease. I have since graduated from this very focused candidate gene approach, and now look for genetic associations with Addison's disease across the PWD's whole genome!

How am I doing this? The very first step is working with people who want to help: I collect lots and lots of samples! Collection efforts largely involve people responding to announcements, newsletters, and Facebook posts about our investigation. I coordinate the sample collection and then work with the collected sample in the lab to extract high quality DNA that is used in different genome-wide sequencing approaches. With the genetic data, I run different computational models to evaluate what regions in the genome may be associated with Addison's

disease. With more samples and time, I will ultimately be able to narrow down the associated region and pinpoint genetic variants that contribute to disease risk—with this, a genetic test for Addison's disease can be created to aid breeding programs and lower the prevalence of disease in our precious breed.

I've spoken to many humans in the PWD community, and it is always so heartwarming to hear about the stories of their dogs who are loved so much, as well as to see how supportive the community is with this genetic investigation. After launching in Spring of 2021, I really enjoyed meeting the faces of many people who had enrolled their dogs in our study. I have gotten to spend time at national specialties, regional specialties, and a water trial! The PWD community welcomed me with open arms; I have learned all about different events and scoring from the friendly faces who walked me through what I was witnessing during the competitions. The pure brilliance of these dogs performing tasks as directed by handlers, along with the goofiness of each pup, has made conducting this research so worthwhile and meaningful to me. I love the breed, the work, and am excited to uncover the risk factors associated with Addison's disease.