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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.

Inside this issue

Lighting the Way: for Hemangio Research

We're pleased to announce a new glassybaby votive candleholder, the idea of longtime Foundation supporter Randi Rossman-Astrom to honor her PWD, Henry.

Seattle-based glassybaby created 'Water Dog Love', a hand-made votive candleholder in honor of all dogs in the fight against hemangiosarcoma.

It takes four artists, three layers of molten glass, two thousand degrees, and 24 hours to make one glassybaby.

Each one-of-a-kind 'Water Dog Love' glassybaby votive has an earth inspired neutrals with an etched silver paw print that will shine with its own glow when lit.

All proceeds from your purchase of 'Water Dog Love' benefit the Foundation's hemangiosarcoma cancer research fund.

For a limited time through April 29, 2019. Pre-Order yours for a donation of $75.

Available exclusively at pwdfoundation.org.

"Puppy Eye Syndrome" (PES)

Margret Casal, DVM, PhD, University of Pennsylvania

Microphthalmia and delayed growth syndrome (aka "puppy eye syndrome" or PES) has been reported by Portuguese Water Dog breeders dating as far back as 1986. The objective of this study is to A) clinicopathologically and molecularly characterize microphthalmia with delayed growth in the Portuguese Water Dog and B) develop a DNA-based test to assist breeders with their breeding programs and avoid producing affected dogs.

Objective A is over 95% complete. A draft of the paper describing the clinicopathological findings had been written but we found more data on affected puppies, which we are currently adding to the paper to be submitted by March 2019. A paper was published about microphthalmia in PWDs last year by a group out of Cornell. However, this paper described only the ocular changes in affected dogs. With our publication, we will show that there can be other abnormalities such as low platelet counts and stunted growth, which makes this a truly syndromic disorder. We have also included pedigree analyses showing the autosomal recessive mode of inheritance.

For Objective B, we received enough DNA samples from affected dogs and their relatives to perform an initial genome wide association study (GWAS). The DNA was aliquoted into plates for the GWAS and was sent to Illumina (Neogen) in November 2018. We received the results as expected in January 2019.

We are absolutely thrilled with the results: A single, very significant peak was seen on the "Manhattan plot", which allows us to locate not only the chromosome but the general area of that chromosome in which the gene must be located. Indeed, there is a gene in this area that, when mutated in mice and humans, causes a microphthalmia syndrome. We are currently in the process of sequencing this gene. If we do not find a disease-causing variant in this gene, we will sequence the entire genome of one affected dog and one normal PWD.

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Final Report: Enhanced Testing for the Diagnosis of Bartonellosis

Edward Breitschwerdt, DVM, North Caroline State University

Bartonellosis, a zoonotic bacterial disease of worldwide distribution, is caused by approximately 10 different Bartonella species. Bartonella are transmitted to canines and humans by ticks, fleas, lice, mites, and sand flies. Dr. Breitschwerdt's laboratory demonstrated the first evidence for Bartonella infections in dogs in 1993. Bartonella species have been associated with an expanding spectrum of important disease manifestations including anemia, endocarditis, hepatitis, lymphadenitis, myocarditis, thrombocytopenia and vascular tumor-like lesions. Infections can be life-threatening. Due to a lack of sensitive and reliable diagnostic assays, definitive diagnosis of bartonellosis in dogs remains a significant problem. Because these bacteria invade cells and infect tissues throughout the body, this chronic intracellular infection is difficult to cure with currently used antibiotic regimens. This study will develop improved serodiagnostic tests for bartonellosis in dogs. These assays can also be used for world-wide sero-epidemiological prevalence studies, and to establish early and accurate diagnosis. Dr. Breitschwerdt's research group has described concurrent infection in dogs, their owners and veterinary workers; this allows for a One Health approach to this important emerging infectious disease.

Report to Grant Sponsor from Investigator

This AKC-CHF funded research has resulted in substantial refinement of our Bartonella serodiagnostic testing knowledge of healthy dogs (pets, working dogs and potential blood donors) and sick dogs being evaluated for a differential diagnosis of Bartonellosis. Based upon testing and comparing results using eight different Bartonella species or strains, we were able to identify two species that when used in combination should increase serodiagnostic sensitivity compared to the three strains that we have used diagnostically for the past decade. As these are not the two species currently in use diagnostically, our historical testing most likely underestimated the prevalence of Bartonella antibodies in seroepidemiological studies published by our research group and more importantly failed to report positive Bartonella spp. serological results that could be used to guide therapy in a sick dog. In both our research and diagnostic laboratory testing, we have always adhered to the adage: The kindest form of therapy is an accurate diagnosis.

This study also allowed us to begin to assess the utility of another serodiagnostic technique called Western immunoblotting for assessment of a dog's exposure to a Bartonella spp. WB interpretation can be challenging and this study has allowed us to compare IFA and WB sensitivities and to define minimal criteria for reporting a "positive" WB. Our efforts to date have identified individual and potential combinations of small Bartonella proteins (peptides) that appear to have diagnostic utility as an inhouse rapid assay that could be used by veterinarians to rapidly determine if a dog has been exposed to a Bartonella spp. We are currently purifying individual proteins and assessing other peptide combinations in an effort to define an assay with optimal sensitivity and specificity. This brief paragraph does not do justice to the numerous hours of research effort that was made possible because of research funding support or the dogs and their owners who will ultimate benefit from these findings, as we improve serodiagnosis of bartonellosis.

Publications and Presentations

Balakrishnan N, Sevala S, Lappin ML and Breitschwerdt EB. Evaluation of Bartonella henselae Western Immunoblotting for canine bartonellosis. Manuscript in preparation.

Neupane, P., Hegarty, B. C., Marr, H. S., Maggi, R. G., Birkenheuer, A. J., & Breitschwerdt, E. B. (2018). Evaluation of cell culture-grown Bartonella antigens in immunofluorescent antibody assays for the serological diagnosis of bartonellosis in dogs. Journal of Veterinary Internal Medicine. 32(6), 1958-1964.

Neupane P, Hegarty BC, Marr H, Maggi RG, Breitschwerdt EB. Evaluation of Eight Bartonella spp. Indirect Immunofluorescent Assays for the Serological Diagnosis of Bartonellosis in Dogs. NCSU-CVM Research Forum, September 2017

Tumor-permissive Collagen Signatures in Canine Mammary Gland Tumors: Development of Prognostic Markers and Targeted Therapies for Improved Outcomes

Susan Volk, VMD, PhD, University of Pennsylvania

End Year 1 Report: Mammary gland tumors (MGT) are the most common malignancies in intact female dogs and recent work indicates that normal, non-malignant cells and extracellular matrix (ECM) within the surrounding tumor stroma regulates the growth and spread of cancer. Our recent study has identified cancerassociated collagen signatures in canine MGT biopsy samples that predict clinical outcome better than commonly used markers. Also, our labs have shown that inhibition of a collagen degrading enzyme (Fibroblast Activation Protein (FAP)) and increasing a tumor-suppressive collagen (type III collagen (Col3)) prevents the formation of these tumor-inciting signatures in other species (mouse and human). Building on these results, we have recently used a novel imaging technique to look at collagen types in MGT samples. Our data suggests that type of collagen as well as the amount and type of collagen cross-linking in tumor samples may be useful in predicting clinical outcome of patients. Based on our published and new data, we predict that identifying and targeting tumor-inciting collagen signatures will improve both diagnosis and treatment of dogs with malignant MGT.

The Role of Complex Translocations Associated with TP53 Somatic Mutations for Aiding Prognosis of Canine Diffuse Large B-Cell Lymphoma

Matthew Breen, PhD, North Carolina State University

End Year 2 Report: This study involves the evaluation of a cohort of canine lymphoma specimens for the presence of tumor-associated abnormalities associated with four key cancer-associated genes (MYC, BCL6, BCL2 and TP53). The presence of these abnormalities, alone and in combination, has been shown to be predictive of the response to standard treatment modalities in human lymphoma patients, and provides powerful opportunities to predict prognosis in newly diagnosed patients. We hypothesize that the same may apply in dogs. We have screened the full cohort of canine lymphoma cases for structural and numerical abnormalities involving MYC, BCL6, and BCL2. Overall the data suggest that rearrangement of the genome at the MYC and BCL6 loci is relatively rare within any given case, and occurs at a frequency similar to what is seen in human DLBCL (Li et al. 2018). While BCL2 rearrangement is highly infrequent in dogs (seen in only 2% of cases), and has a generally neutral copy number status, our initial analysis suggests an association with disease-free interval. In an earlier study we showed that the incidence of BCL2 rearrangement and copy number imbalance is low in canine follicular lymphoma (Thomas et al. 2017). The rarity of this B-cell lymphoma subtype in the dog limited the ability to draw generalized comparisons with the human counterpart; however the present study suggests that these observations can be extended to other more common canine B-cell lymphomas. Analysis to date suggests that neither BCL6 nor MYC rearrangement is significantly associated with disease free interval. Assessment of the copy number status of both of these loci concur with previous studies (Thomas et al. 2011), with MYC demonstrating a slight copy number gain and BCL6 demonstrating largely neutral copy number status. DNA sequencing analysis of the TP53 gene has revealed a diverse series of variants among those cases analyzed to date, the majority of which are clustered within a small genomic interval. Almost all variants are simple in structure but are predicted to have a deleterious effect on the function of the gene. We identified variants for which the equivalent alteration is highly recurrent in human tumors, including two key variants that have been reported previously in canine lymphomas, adding to their potential clinical significance.

Targeting the Cancer Epigenome: The Effect of Specific Histone Lysine Methyltransferase Inhibition in Canine B-Cell Lymphoma

Angela McCleary-Wheeler, DVM, PhD, University of Missouri

End Year 2 Report: Lymphoma, particularly the large, B-cell subtype, is one of the most common malignancies in dogs. Canine lymphoma can be treated, but it is rarely cured. Novel therapeutic strategies are necessary to improve outcomes in dogs diagnosed with lymphoma. Recently, advances in the understanding of human lymphomas have focused on the area of epigenetics. One area of this research involves understanding how genes are turned on or off based on different modifications to histone proteins, a specific group of proteins that interact with DNA. Specific enzymes that modify these histone proteins have altered activity that can lead to lymphoma development in human lymphomas. One of these enzymes is EZH2. Increased activity of EZH2 has been shown to play an important role in the development of some human lymphomas. Very recently, data from a Phase I study of the EZH2 inhibitor, tazemetostat, in relapsed or refractory human B-cell, non-Hodgkin lymphoma has shown to be a safe, oral therapy with potential clinical benefit. The role of EZH2, however, has not been evaluated in canine B-cell lymphomas to date. Given the similarity between human and canine B-cell lymphoma, we sought to investigate whether EZH2 activity plays a role in canine B-cell lymphoma. To do this, we use canine lymphoma cells and specific EZH2 inhibitors, including the tazemetostat used in early human studies, to evaluate the effect of EZH2 inhibition on cell growth and survival. Our data suggest that this inhibitor is highly potent and effective for inhibiting EZH2 effects on histones in canine lymphoma. This is important as this inhibitor is an orally bioavailable drug with a good toxicity profile in humans, making this inhibitor a candidate for clinical trials in dogs with lymphoma. Initial data suggests that EZH2 inhibition may not impede lymphoma cell proliferation or survival. However, evaluation of the genes EZH2 regulates is needed to understand why this is the case. We have confirmed that some genes that regulate the ability of canine lymphoma cells to replicate are altered with EZH2 inhibition. Specifically, one gene, CDKN1a, is turned back on when EZH2 is inhibited. The activation of CDKN1a is repeatable and profound. We will be continuing this work with a sequencing approach to further understand what genes are regulated by EZH2 in canine B-cell lymphoma. Our findings are suggesting an importance for EZH2 in canine lymphoma and for continued investigations into cell cycle regulators that may be abnormal. We are encouraged by the data thus far and look forward to evaluation of sequencing results. We are also continuing this work with two newly developed canine B-cell lymphoma cell lines – a major development for researchers

Surveillance of Hepatozoon americanum in Populations of the Gulf Coast Tick

Andrea Varela-Stokes, DVM, PhD, Mississippi State University

End Year 1 Report: American Canine Hepatozoonosis (ACH) is a protozoal disease in dogs caused by Hepatozoon americanum. This organism is transmitted to dogs when they ingest the definitive host for the protozoan, the Gulf Coast tick (Amblyomma maculatum; GCT) or a paratenic host (rodents and rabbits). There is currently no treatment for eliminating protozoa in the infected canine. The main source of infection, tick or paratenic host, has not yet been identified for ACH making it difficult to develop preventative measures. Data on disease prevalence and distribution are reliant on detection in the affected canine. The geographical distribution of ACH is assumed to overlap GCT distribution. Currently, there is no data detailing H. americanum prevalence in the tick vector. This study aimed to fill the gap in knowledge by investigating infection prevalence in adult GCT. This tick is active in summer months. In 2018, we collected 129 adult GCTs from 3 different sites in Oktibbeha Co., MS and extracted DNA from half or whole ticks. We used a TaqMan quantitative PCR assay to test for H. americanum. No half tick extracts were positive using conservative threshold levels; two whole tick extracts had low copy numbers but could not be confirmed. To evaluate potential false negative extracts, ticks with amplifiable DNA were tested with conventional PCR. No ticks were confirmed positive. Thus far, results suggest GCTs in this area may not be a primary source of H. americanum infection in dogs. Future studies targeting areas with ACH cases and investigating infection rates of nymphal GCTs and various paratenic hosts will offer more insight on the main transmission route for ACH here. This information could be crucial for the development of improved methods for prevention.

Lyme Disease in Dogs: Prevalence, Clinical Illness, and Prognosis

Jason Stull, VMD, PhD, The Ohio State University

Mid-Year 3 Report: Three US veterinary clinics across the gradient of Lyme endemicity along the East Coast (2 clinics in Maine, 1 in Western Pennsylvania) have participated in the study. Since June 2017 the three clinics have been collecting and providing to the study team data on all dogs tested with an antibody test for B. burgdorferi (including dog signalment, B. burgdorferi and co-pathogen results from current antibody test, and prior results for B. burgdorferi and co-pathogen screening). For two of the clinics, data collection ceased in July 2018, with testing data on 1,800 dogs (clinic 1) and 600 dogs (clinic 2) provided to the study team. Data collection for the third clinic was closed on December 31, 2018, with data on 2,400 dogs reported to the study team. These data (results for a total of 4,800 dogs) are now being analyzed to determine the seroprevalence of B. burgdorferi in dogs living in regions of the US with varying levels of infected-tick exposure risk and to identify important factors associated with dogs testing positive for B. burgdorferi. Approximately 11% of these dogs have had a current positive B. burgdorferi result, of which 6% were also positive for Ehrlichia and 18% positive for Anaplasma. These data are consistent with the study team's expectations. At the time of testing, 2 of the clinics have also been enrolling owners of test-positive and test-negative dogs into the survey component of the study. To-date, over 400 dog owners have completed the initial survey (~25% of which had a dog with a current B. burgdorferi-positive test). This proportion of positive dogs is as planned. After completion of the initial survey, dog owners are invited to take additional surveys 3 and 6 months later (to assess for dogs' clinical signs and owners' changes in tick prevention practices). To-date, over 180 and 100 dog-owners have completed the 3- and 6-month follow-up surveys, respectively.

Genetic and Environmental Risk for Lymphoma in Boxer Dogs

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

End Year 2 Report: Lymphoma is a fatal cancer of the blood cells that can occur in any dog. Lymphoma is more common in Boxers, Golden Retrievers, and several other purebreds, which suggests involvement of inherited genes. Recent research has focused on gene mutations in the tumors of dogs with lymphoma. However, we do not understand why these mutations accumulate in certain dogs, and this understanding is essential for disease prevention. Canine lymphoma resembles Non-Hodgkin lymphoma (NHL) in humans, which is more common in industrialized countries and is associated with chemicals found in tobacco smoke, certain household products, pesticides, herbicides, and fungicides. Glutathione-S-transferases (GSTs) are enzymes that can break down toxic chemicals in the body and prevent tumor mutations. Inherited gene defects in the 3 major GST enzymes, GST-theta, GST-pi and GST-mu, each increase NHL risk, and simultaneous defects in more than one enzyme further increase NHL risk. The investigators have characterized two GST-theta enzymes in dogs, and both have defective gene variants. So far, their findings suggest one variant is a risk factor for lymphoma in dogs of varying breeds. However, the genes for canine GST-pi and GST-mu enzymes have not yet been explored. This research will determine whether defective GST genes along with certain household and yard chemicals are associated with lymphoma in dogs, with a focus on the high-risk Boxer breed. The overall goal of this study is to identify combinations of genes and environmental chemicals that contribute to the development of lymphoma in dogs, so that better cancer prevention strategies can be developed.

GST genes defend against environmental chemicals that could cause cancer. Our goals for this study are to determine whether defective GST genes are more common in the boxer breed, which has a high risk of lymphoma, and are associated with lymphoma in boxers in combination with environmental exposures.

Defining the Mechanism by Which Ticks Locate Dogs in Order to Better Prevent Disease Transmission

Emma Weeks, PhD, University of Florida

The brown dog tick (BDT) is common across the US and the most widely distributed tick in the world. BDT's are capable vectors of pathogens that cause canine ehrlichiosis and babesiosis as well as other disease agents. Prevention of these diseases is accomplished through tick control. BDT's can complete their entire life cycle indoors, making management difficult. Records of infestations are increasing and unpublished data indicates that a high level of acaricide resistance is present in domestic populations. Consequently once introduced, these ticks are particularly hard to eradicate and as one female tick may lay 5,000 eggs, the problem soon gets out-of-hand. Acaricide resistance leads to aggressive treatment regimes, which in turn, leads to increased exposure of humans and pets to acaricide residues. Alternatives to pesticide applications are needed. Studies have shown that BDT's are attracted to dog odor, a blend of volatile chemicals used by ticks to find a blood meal. (*Continued on page 8*)

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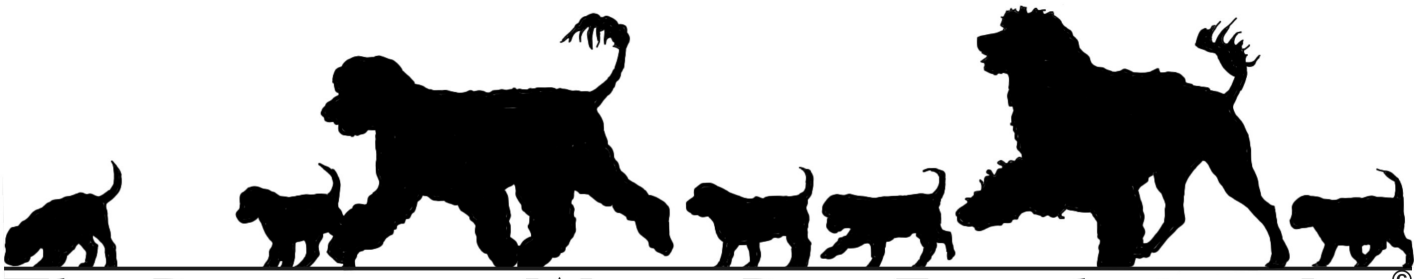

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Portuguese Water Dog Club of the Twin Cities in memory of the PWDs we lost in 2017

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Candi & Chuck Bubert in honor of "Luna" Aspen Coves Lunar Shadow TD THDA AWD, a phase 3 participant in the Shine On Project

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Roberta Capuano & Tom Heffernan in honor of Jane Freeman for the gift of "Dutch", "Piper" and "Jibe"

Roy & Barbara Cawley in loving memory of Ruth Henderson and her special girl "Crystal" CH Makitso's Crystal Gale

Rick & Cindy Eastman in memory of "Elly", forever in our hearts and loving memories

Fred & Susan Forman in memory of "Molly Brown" Cypress Bay's Unsinkable Molly Brown

Verne Foster in memory of dogs we have loved and lost

Jenn & Mike Greene

Martin Hatlie in honor of the PWD PSG

Melinda Reid Hatton in memory of Layla and in honor of Lilly

Thomas & Linda Majcher in congratulations on your new title to "Liam" GCH CH Allegiance Love 'Em Or Liam for his grand championship!

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Diana Bailey wishing Happy Birthday to Summer Pups

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Vicki Goldberg in honor of all my healthy grandchildren & children

Angela Harding in memory of "Roxie" Int/Am/Can CH RainCity's All That Jazz CD BN RE JWD CGC MAC-1

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Colleen Lemasters in honor of Aidan

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Pamela Marshall

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Kenneth & Katherine Tallering

Catherine Tinaglia in honor of Rixa and Louise Mowbray

Dolores Torriero

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Marcia Wallace & Paul Zoschke in thanks to Alan Smucker

Thomas & Lauren Wallitsch in memory of Regina "Reggie" Wallitsch

Anne Colston & Dennis K. Wentz

Gary & Sandi Willis in honor of Bella & Toli, forever in our hearts!

Penny Yamamoto

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Anonymous

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Ann Benninger in honor of the PWD PSG

Curt & Mary Conover as a Christmas gift to the Coniglio family & Libby

Paul Croce in memory of Bobbi Croce and Sweetie Pie

Patricia Cronin

John & Susan Cucura in memory of "Maya" CH Freestyle Someone Like You

Rachel Eken in honor of the PWD PSG

Ordean & Dorothy Finkelson in memory of our grand puppy "Moby" Kalista Lands A Lunker VCD1 BN RAE MX MXJ TKA WWD GROM. We miss your sweet & gentle soul and fun-loving personality. Rest in peace.

Ordean & Dorothy Finkelson in memory of Neptune, Osha and Moby

Barbara Floch in honor of the PWD PSG

Amanda Ford in memory of Pumpkin Myrick

Thomas N. Foster III

Jose Franceschi in honor of the PWD PSG

Angela Harding in honor of the PWD PSG

Christine Harris in honor of the PWD PSG

Michele Hemenway

James & Patricia Hobin in loving memory of "Nettie" Cutwater Capture My Heart AX AXJ OF WWD

Jayne Hopkins in honor of the PWD PSG

Patrice Horstman

Linda K. & Krista K. Hunt, Kalista in memory of Mary-Margaret Moreau, dear friend and PWD lover

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Kuper" CH Kalista's Terceiro NA NAJ for his NA and NAJ!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Mini" Kalista's Wanna Keeper NA NAJ for her NA and NAJ!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new titles to "Krickie" Kalista's Knee Jerk Reaction RN TKA SCN SIN THDD CGCA for her SCN and SIN!

Linda K. & Krista K. Hunt, Kalista in honor of Deb Scofield & "Krickie" Kalista's Knee Jerk Reaction RN TKA SCN SIN THDD CGCA for their teamwork and dedication to Therapy work. Congratulations on "Krickie's" THDD!

Sandy Iwaszko in honor of the PWD PSG

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Susan Kues in memory of Molly the Wonderful Bull Terrier

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Barry & Suzette Pangrle

Sue Pemberton in honor of the PWD PSG

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Mary Sebera

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Elaine Suter in honor of the PWD PSG

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Michelle Walby in memory of Pooch

Janet Warnsdorfer, Galaxy PWDs in memory of "Elmo" CH Galaxy's St. Elmo's Fire

Bob & Yukie Wester in memory of "Charlie" Torrid Zone Jump To The Rhythm CGC TKI

Bob & Charlene Wolfe in loving memory of Rainy

Deck Hand up to $49

Anonymous

Linda Carey in memory of "Misty" Rockmere Sea Mist, loved and missed by the Brown family

Carol & Warren Cooke in memory of Randy Latham at Christmas

Carol & Warren Cooke in memory of Cole. He was a very special boy!

The Ekl Family in memory of "Cosmo" loved and missed by the Tucker Family

John Haeger in memory of "Rudy" Crews'n Ports Rudolph's Glo CD

Carol & Howard Hilman in honor of Riva & Hildie the best companions

Sandy Kott in memory of "Ziva" Bantry's Pool Dolphin MX MXJ XF WWD

Linda K. & Krista K. Hunt, Kalista in memory of "Moby" Kalista Lands A Lunker BN RAE VCD1 MX MXJ TKA WWD GROM, loved and missed by Kristi & Mike Portugue, Hydro and Sprite

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Sunni" Am/Can CH Kalista's Sun Star Traveler RE NA NAJ WWD for her WWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Zora" Kalista's Zora The Duck Explorer AWD for her AWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Tally" CH Kalista's What A Catch TKI WWD for her WWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Polo" Kalista's Pursuit of Happiness AWD for his AWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Lyra" Kalista's Vega A Lyrae RN TKI AWD for her AWD!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Hydro" CH Kalista's To Splash-N-Dash BN RN TD MX MXJ T2B TKI CGC WWD for his WWD!

Linda K. & Krista K. Hunt, Kalista in memory of "Queixo" MACH3 Kalista's Just What The Dr Ordered UD RAE FDC MXG MJB2 MJP2 MFB MFP T2B CGC TKI CWDX SROM, loved and missed by Tiffani Flaws

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Beamer" GCH CH Kalista's Ultra Black Magic Water RN MX MXJ OF TKI CGC for his MX!!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Tally" GCH CH Kalista's What A Catch WWD TKI CGC for her GCH!!

Linda K. & Krista K. Hunt, Kalista in congratulations on your new title to "Y" MACH3 PACH Kalista's Now What CD BN RA FDC MXC MJB2 MJP3 MJPB MFB T2B CAX BCAT TKI CWDX CGC for his PACH!!

Kate Jackson in honor of Joan Bendure and Dr. Laura Carey, LVM

Debbie & David Minkoff

Susan Myrick in memory of "Molly Brown" Cypress Bay's Unsinkable Molly Brown UD VER RA WWD

Ashley Reid on behalf of Patty McDowell

Kathleen Skeels

Southern California PWD Club in memory of "Roxy" CH Seashell's Surfer Girl CGC JWD AOM

Southern California PWD Club in memory of "Gus" Deerpark's Magic Gus

Jennifer Turner in memory of Bella

Mary Warner

(Continued from page 5)

Identification of the chemicals BDT's use to locate a dog (semiochemicals) would enable manipulation of tick behavior thereby facilitating management and reducing the need for extensive use of acaricides. Improved tick control without the need for increased acaricide applications will improve the quality of life for dogs and their owners or handlers. Work will be accomplished through four successive objectives to 1) collect dog odor, 2) identify chemicals that ticks can detect, 3) test chemicals for tick attraction and ultimately 4) evaluate efficacy of an attractant-based tick trap.

For the first objective, the collection of dog odor, all animals have been identified and the samples have been collected and analyzed by chromatographic techniques. Furthermore the chemicals have been identified tentatively by mass spectrometry. For the second objectives the electrophysiological techniques have been established and ten ticks have been tested against each dog breed sample plus a mixed sample of all dog breeds (Total 60 ticks). Comparisons between the electrophysiological responses by breed have been made and those peaks producing consistent responses in tick sensory organs have been identified. For the third objectives the behavioral assay has been established. Attraction has been demonstrated to whole dog hair samples and positive controls. Further studies will test the electrophysiologically active chemicals in the behavioral bioassay to determine behavioral role and impact. The most attractive chemicals will be tested in a semi-field trapping system for potential use in a monitoring device.

Developing a Next Generation Sequencing Diagnostic Platform for Tick-Borne Diseases

Pedro Diniz, DVM, PhD, Western University of Health Sciences

Mid-Year 1: Diagnostic tests based on the detection of DNA from harmful organisms in clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several vectorborne diseases (VBDs) are available through universities and private labs in the USA and abroad. However, the vast majority of results from sick dogs are negative, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis. These panels are based on the detection of previously known DNA sequences of each pathogen, which limits their ability to detect novel organisms. Using an innovative approach, our study proposes the adaptation of high-throughput nextgeneration sequencing (NGS) to the detection of tick-borne bacteria in dog blood to overcome the limitations of the current diagnostics. NGS is capable of generating millions of individual sequencing reads from each sample, allowing for the unbiased identification and characterization of multiple organisms from a single sample. We are pioneering this strategy in the Veterinary Medicine VBD diagnostics field, and important results were already achieved from our previous AKC-CHF grant (#02292). Since we are dealing with a cutting-edge technology, our work is under continuous and systematic adjustments, aiming enhancements in the platform in order to accurately detect infected dogs and precisely determine which bacteria are responsible for disease. In this current first report of grant #02528, we describe our bioinformatics efforts on comparing the 'state-of-the-art' computational tools using dog blood samples as input, as well as provide a comprehensive standard operating procedure (SOP) for best practices of microbiome analyses applied to VBD diagnostics, as part of the specific aim 3 (SA#3). In parallel, as part of SA#2, we have also started a search for a new marker gene (other than 16S rRNA) through computational screening of whole genomes in order to achieve a better discriminatory power on the taxonomic classification of VBD causing bacteria, thus increasing the diagnostics capability of detecting species and strains. Regarding improvements on benchtop microbial DNA isolation techniques (SA#1), we have performed initial tests, which have demonstrated to reduce host DNA concentration in infected dog blood samples confirmed by quantitative PCR assay. Our results are in line with the proposed timeline. We truly believe that our ongoing AKC-CHF research will support the development of better diagnostic tools that will simultaneously advance both canine and human health.

ADDITIONAL INFORMATION ABOUT NEWLY FUNDED RESEARCH GRANTS AND PROGRESS REPORTS ON PREVIOUS GRANTS ARE AVAILABLE AT OUR WEBSITE:

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