

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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MOU for PES Research

In collaboration with the PWDCA, the Foundation will fund research into PES at UPenn.

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Shine On: Hemangio Research Update

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AKC CHF and MAF Progress Reports

Updates on research we've co-sponsored. More detailed information is available on our website.

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Donation Form

Please consider donating to the Foundation. Every dollar we receive helps us fund critical medical research. Thank you!

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Honor Roll of Donors

Our list of donors who contributed between July and December 2016.

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PWDF DONATIONS EXCEED \$1,000,000

The Foundation has reached the incredible milestone of \$1,000,000 in donations. Our "one millionth" dollar came from Linda Hunt, a financial founder and long-time donor. Linda's dedicated support of the Foundation is inspired: every time a dog she has bred earns a title she makes a donation in the dog's name. She also regularly acknowledges the achievements and passing of her dogs and human friends with memorial tributes, and she contributes to major campaigns, such as Shine On and PES. Her generosity is greatly appreciated.

Currently, the Foundation holds \$518,000 — perfectly positioning us to fund future breed-specific and joint research as it becomes available. Since 1998, we have approved \$580,935 dollars in funding. Thanks to donations like yours and Linda's, we have been a major contributor in the development of the DNA gene test for Juvenile Dilated Cardiomyopathy, as well as the test for Progressive Retinal Atrophy. The Foundation sponsors studies on cancers, Addison's disease, Inflammatory Bowel Disease, hip dysplasia as well as other important areas of health research.

PUPPY EYE SYNDROME: PES

For the past 9 years, the Foundation has supported early data gathering for Puppy Eye Syndrome (PES), collaborating with researchers, veterinarians, breeders and volunteers. We greatly appreciate everyone who has supported this endeavor, and are delighted that the Foundation has signed a Memorandum of Understanding (MOU), along with the Portuguese Water Dog Club of America, to collaborate with AKC CHF to jointly fund research at the University of Pennsylvania (UPENN).

The goal of this research is to better characterize the clinical disorder of syndromic microphthalmia and delayed growth seen in the PWD, commonly known in our breed as Puppy Eye Syndrome (PES). The goal of this research is to confirm the suspected mode of inheritance, to

continue to obtain research samples, and to investigate the genetic cause for this disorder in PWDs as well comparative research in humans.

To help us fund this research, Peggy Helming and Milan Lint offered the Foundation a matching gift challenge of \$10,000 during the month of February.

We were able to double the impact of your gifts and not only reach, but exceed our goal of \$10,000. In just over 8 days, you donated more than \$10,000, which was generously matched by Peggy and Milan. Thank you to everyone who contributed to this important cause. A list of donors is on page 7 and 8.

This MOU is a first step: further details will be available once this grant has undergone the peer review process.

The Board of Directors welcomes Kara Kolster, DVM, DACT, a graduate of the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) at Virginia Tech. In 2007, she completed a residency in Theriogenology (reproduction) at VMRCVM. Dr. Kolster practices at the Springfield Veterinary Center in Glen Allen, Virginia.

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AKC CHF Progress Reports

Investigating a Ketogenic Medium-Chain Triglyceride (MCT) Supplement for the Treatment of Drug-Resistant Canine Idiopathic Epilepsy and Its Behavioral Comorbidities*Dr. Holger Andreas Volk, DVM, PhD; Royal Veterinary College, University of London*

Canine epilepsy is a chronic neurological condition, often requiring lifelong medication with antiepileptic drugs (AEDs). Despite appropriate treatment with available AEDs, seizure freedom may not always be achievable. Indeed, over two thirds of dogs with epilepsy continue to have seizures long-term and around 20-30% remain poorly controlled on standard AEDs. The hardest to treat dogs are termed 'refractory' or 'drug-resistant' patients. There is an urgent need to improve the quality of life (QoL) of drug-resistant patients. The ketogenic diet, originally characterized as high in fat and low in carbohydrates, has been a successful treatment in children with epilepsy for several decades, decreasing seizure activity and even leading to seizure freedom in drug-resistant patients. Recent research has identified that a component of the ketogenic diet, a medium-chain fatty acid (MCT) called C10 has direct anti-seizure effects on the brain. The investigators will assess whether dietary supplementation with ACT oil containing C10 for dogs with drug-resistant epilepsy will reduce seizure frequency and/or severity. As epilepsy has multiple impacts on QoL beyond seizure frequency the researchers will also investigate whether the MCT supplement alters the side effect profile of AEDs, improves behavioral problems associated with epilepsy (e.g. anxiety) and cognition, and improves the stress levels of the affected dog. If successful, MCT supplements could provide a new tool for canine epilepsy treatment.

Identification of Genetic Risk Factors for Canine Epilepsy*Dr. Gary S. Johnson, DVM PhD; University of Missouri, Columbia*

Epilepsy is one of the most common neurologic diseases of dogs and a top concern of dog breeders. Despite strong evidence that genetics is important in determining the risk of idiopathic epilepsy, numerous gene mapping studies have failed to identify a locus that accounts for that risk in either dogs or humans. Seizures occur when excessive activity goes beyond the normal threshold for brain function, many factors contribute to that level of activity, and therefore, mutations in numerous genes may

collectively contribute to increased activity until that threshold is exceeded, resulting in epilepsy. Any one of these mutations may be present in non-epileptic dogs, but because it only partially alters activity, it would not produce seizures. Therefore, traditional gene mapping studies might overlook that mutation.

Using a novel whole genome sequencing approach the investigators hope to identify DNA variations in epileptic dogs that could affect the function of genes such as ion channels and neurotransmitter receptors that have been shown to alter the seizure threshold in humans or rodents. The frequency of such variations in populations of epileptic and non-epileptic dogs will be directly compared rather than the indirect markers used in traditional mapping studies. The increased power provided by looking for specific gene candidate variations rather than linked markers will aid the identification of epilepsy risk factors, perhaps leading to development of DNA tests to enable breeders to select against such risk factors.

Disease Risks Associated with Spay and Neuter: A Breed-Specific, Gender-Specific Perspective*Dr. Benjamin L Hart, DVM, PhD; University of California, Davis*

This study extends the investigator's recently completed AKC Canine Health Foundation-funded project studying 12 dog breeds to identify major differences in the degree to which spay or neuter may be related to an increase in joint disorders (hip dysplasia; cranial cruciate ligament tear) and/or cancers (lymphoma; hemangiosarcoma; and mast cell tumor). The original breeds studied were: Labrador Retriever, Golden Retriever, German Shepherd Dog, Rottweiler, Boxer, Bulldog, Doberman Pinscher, Dachshund, Corgi (both breeds), Chihuahua, Yorkshire Terrier and Shih Tzu. Findings did not associate an increase in disease association in the small breeds with spaying or neutering, while in larger breeds disease risk was dependent upon gender, and whether the spay or neuter procedure was performed before or after one year of age.

Upon completion of the study, the major publisher, Wiley, has agreed to place the total data set of all 31 breeds on an open access website as a resource for breeders, dog owners, researchers and veterinarians.

Broad-Range Detection of Canine Tick-Borne Disease and Improved Diagnostics Using Next-Generation Sequencing

Dr. Pedro Paul Diniz, DVM, PhD; Western University of Health Sciences

Diagnostic tests based on the detection of DNA of infectious organisms from clinical samples have revolutionized veterinary medicine in the last decades. Currently, diagnostic panels for several tickborne organisms are available through universities and private laboratories in the USA and abroad. However, the vast majority of results from clinically ill dogs are negative for tick-borne diseases, which frustrates veterinarians and dog owners trying to reach a definitive diagnosis and improve treatment options. These panels are based on the detection of previously known DNA sequences of each pathogen, with little room for detecting new organisms. Consequently, the current assays may suffer from "myopia": a self-fulfilling effect that prevents the detection of new or emerging organisms. Using an innovative approach, the investigators will employ next-generation sequencing (NGS) to overcome the limitations of current diagnostic technology. With NGS, the investigators can generate millions of individual gene sequencing reads from each clinical sample, allowing for the identification and characterization of multiple organisms from a single sample. Testing samples from dogs naturally exposed to tick-borne diseases, NGS will detect not only new organisms but also characterize genetic differences among known organisms. The resulting dataset of a large number of DNA sequences of known tick-borne organisms and previously undetected organisms in naturally-infected dogs will support the development of diagnostic tools to simultaneously advance canine and human health.

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

Dr. Matthew Breen, PhD; North Carolina State University

Lymphoma accounts for up to 24% of all cancers diagnosed in pet dogs. Among these cases diffuse large B-cell lymphoma (DLBCL) is the most common subtype veterinary medicine, the response to treatment for canine lymphoma remains highly variable with no reliable means to predict response. Studies of lymphoma in

people have identified characteristic genome changes that have both diagnostic and prognostic significance. In human DLBCL, mutations in the TP53 gene, and genome rearrangements involving the MYC, BCL2 and BCL6 genes have been shown to confer particularly poor

prognosis in cases treated with standard of care multi-agent (CHOP-based) chemotherapy. The investigator's previous CHF-funded studies have shown that canine cancers, including lymphoma, exhibit genomic changes that are conserved with those observed in the corresponding human cancers, and have identified MYC and BCL2 rearrangements and a high frequency of TP53 mutation in canine DLBCL. This research will screen a well-defined collection of over 450 pre-treatment, canine DLBCL samples to determine accurate frequencies of these genome changes. The researchers will investigate the correlation of these target aberrations with duration of first remission, and identify key genomic signatures that may aid prognosis of prospective canine lymphoma cases. The data generated should assist owners and veterinarians with decisions regarding treatment with CHOP. Patients with signatures predictive of poor response to conventional CHOP chemotherapy may benefit from more aggressive treatment at the outset to improve outcome.

Genetic and Environmental Risk for Lymphoma in Boxer Dogs

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

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.

MAF Progress Reports

Measuring chemotherapy drug resistance in dogs with T-cell lymphoma

Paul R. Hess, DVM, PhD, North Carolina State University

Morris Animal Foundation-funded researchers from North Carolina State University are using state-of-the-art DNA technology to improve the outcome of dogs with T-cell lymphoma. Although most dogs with T-cell lymphoma temporarily attain remission when treated with chemotherapy, small numbers of drug-resistant cancer cells remain. Measuring the changing levels of these cancerous T-cells could provide valuable new information on the effectiveness of different chemotherapy agents, allowing for tailored treatments for individual patients. The research team first studied the diversity of T-cells in the blood of both normal and T-cell lymphoma dogs to establish a robust baseline for chemotherapy-treated dogs for the study. Four canine lymphoma patients are now enrolled in the clinical trial and all dogs are receiving standard-of-care multidrug chemotherapy.

In the coming year, the team aims to recruit additional T-cell lymphoma canine patients and complete all sample collection and processing for final analysis. Ultimately, the research team hopes to provide veterinary oncologists with the tools needed to treat dogs with T-cell lymphoma using patient-customized, adaptive chemotherapy protocols that should result in longer remission and survival times.

Mapping of Genetic Risk Factors for Canine Hip Dysplasia

Dr. Antti Iivanainen, DVM, PhD; University of Helsinki and the Folkhälsan Institute of Genetics

The overall objective of our study is to perform a genome wide association study (GWAS) of canine hip dysplasia (CHD) in German Shepherds using a large sample cohort (200 cases and 200 controls). CHD is a common problem in many breeds. The dysplasia phenotype is graded from radiographs. In this study, we use the standards of Fédération Cynologique Internationale (FCI) ranging from A (healthy) to E (severely dysplastic). Each hip joint is graded individually. As the disease progresses also the risk for hip joint arthrosis -- a painful and incurable condition -- increases. The identification of genetic risk factors would enable the development of genetic tests to aid the breeders in controlling the disease. Four hundred animals consisting of carefully matched pairs of healthy and affected individuals should provide enough power for the association study to uncover the major genetic risk factors for this degenerative disease.

At present, we have collected a study cohort of 1141 dogs including 411 cases and 730 controls. We have analyzed the association of CHD to a genome wide array of genetic markers using 526 dogs. The results indicate suggestive associations to ca. 30 markers on several chromosomes. Preliminary analyses suggest that CHD associates with some of the markers also in an independent cohort of 833 dogs from four breeds. We are currently finalizing the analyses and preparing a manuscript on the results.

Exploring New Medical Treatments for Cushing's Syndromes in Dogs

Sara Galac, DVM, PhD, Utrecht University

Hyperadrenocorticism, also known as Cushing's disease, is one of the most common endocrine diseases in older dogs. The disease develops when the body produces higher than normal levels of the hormone cortisol. Dogs with hyperadrenocorticism often are managed medically with drugs to control clinical signs. Although many therapeutics are effective, the drugs sometimes have severe side effects, highlighting the need for newer, safer therapies.

Morris Animal Foundation-funded researchers from Utrecht University, in the Netherlands, are exploring novel treatment options for dogs with hyperadrenocorticism. The elevated cortisol levels associated with this disease are triggered primarily by pituitary gland and adrenal gland tumors. Using tissue cultures derived from canine cortisol-secreting adrenal tumors, the research team is assessing the effect of several novel compounds on cortisol production and adrenal tumor growth.

Preliminary results are promising. The research team is incubating adrenal cells with several therapeutics that fall into two broad categories of compounds. Preliminary data on the first set of samples show a decrease in cortisol concentration in adrenal cells after treatment. However, further data are needed to confirm these results. During the next year, the research team will continue to recruit dogs into the study to obtain additional tissue samples for analysis. The research team also will collect data on how the novel compounds affect tumor growth and cortisol synthesis.

Few treatment options are available for dogs with adrenal gland tumors, largely because the mechanism by which the tumors release excessive cortisol is poorly understood. This study is filling knowledge gaps about the biological behavior of adrenal tumors while exploring options to improve treatment strategies for dogs with hyperadrenocorticism.

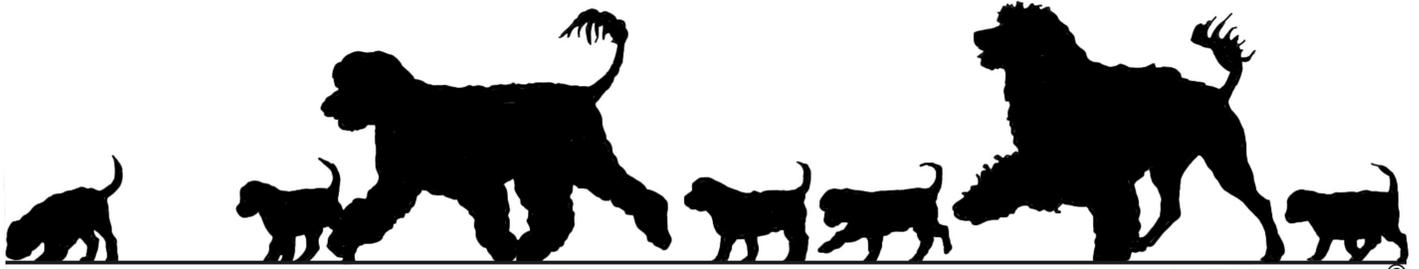
A Novel Approach for Prevention of Canine Hemangiosarcoma

Dr. Jaime F Modiano, VMD, PhD; Research Institution: University of Minnesota

Hemangiosarcoma is the cause of death for one out of every five Golden Retrievers in the United States. Portuguese Water Dogs and Boxers also have an especially high risk for this disease. This disease is incurable partly because it is detected at a very advanced stage when it is resistant to conventional therapies. Thus, an unconventional approach to improve outcomes for hemangiosarcoma would involve effective methods for early detection and for disease prevention. For this project we will do precisely that by pairing two novel technologies consisting of a patented test to detect hemangiosarcoma cells in blood samples and a treatment that attacks the cells that establish and maintain the disease. We will accomplish three milestones: first, we will

expand our understanding of the performance of the blood test in dogs with active disease. Second, we will confirm the utility of the test to predict progression of the disease in treated dogs. And third, we will establish the performance of the test in the "early detection" setting (dogs at risk without evidence of active disease), and hemangiosarcoma prevention through eradication of the tumor initiating cells with the targeted drug. At the end of this project, we expect to have created tools to guide further development, licensing and deployment of these paired technologies for cancer prevention in the community setting. Over the past 6-months, we have made substantial progress toward objective-1, "To confirm that our patented test can detect hemangiosarcoma cells in the circulation prior to the onset of grossly detectable disease." Specifically we have:

1. Verified that if we add hemangiosarcoma cells from tumors we have cultured in our laboratory to blood samples from unaffected dogs that are healthy and do not have any evidence of having hemangiosarcoma, other cancers, or other diseases (what we call spiking), we can detect those cells using our patented assay with currently available reagents
2. Established a threshold for detection of these "spiked" hemangiosarcoma cells. In other words, the smallest number of cells that must be present in a blood sample in order for us to detect them. This is done per unit of measure, for example cells per microliter, where a standard drop of blood equals about 20-30 microliters
3. Confirmed that there is a direct relationship between the number of cells spiked into a blood sample and the number of cells we detect in the assay
4. Advanced confirmation that the markers used for our test do not detect cells in blood of dogs with non-malignant splenic disease (nodular hyperplasia with hematoma)
5. Advanced confirmation that the markers used for our test do not detect cells in blood from dogs with other cancers (osteosarcoma and lymphoma)
6. Identified technical obstacles that need refinement in the test (antibody interference)
7. Started a systematic search for other targets using our RNAseq databanks
8. Started testing a new robotic system to enrich circulating tumor cells from blood samples.



The Portuguese Water Dog Foundation, Inc. ©

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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 tax-deductible 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.

Donor levels are: Deck Hand (up to \$49) - Sailor (\$50 to \$99) - Boatswain (\$100 to \$249) First Mate (\$250 to \$499) - Captain (\$500 to \$999) - Commodore (\$1,000 or more)

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HONOR ROLL OF DONORS

Thank you to all of you who support the efforts of The Foundation. We appreciate every dollar you donate. And we still have much to accomplish. This list includes people who contributed between July and December 2016.

Commodore \$1,000 and up

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Elizabeth Carl & Victoria Hill in memory of Windsong's Callahan "Jonah"
Estate of Lucia Brown Dudley, deceased
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Geri Zuckerman, Seadream Portuguese Water Dogs

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Kathy Lichtenstein in memory of my best guy, Rex Cooper. Missing you always.
Tom & Karol Mauss
Sheilah & Peter Phelan in memory of "Lucy" Saltydawg's Perfect Storm
Joan Sennett
United Sunshine State PWD Club from proceeds of the basket raffle held at the annual meeting and holiday party
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Mountainhead's Luxo DeGifford missed and loved by Connie Szefflinski & Hale Landis

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Delmarva PWD Club in thanks to Cheryl Phillips for all of her hard work and countless contributions to the club

Great Lakes PWD Club in honor Keel Tonel

Great Lakes PWD Club in honor of the 2016 Specialty

Cheryl Winchell Hoofnagle in memory of Dante

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Pacific Northwest PWD Club thanks Dave & Rebecca Sliger for hosting Agility Fun Day

Elaine & Mark Suter in memory of CH Hunter's Black Tagus NA OAJ JWD

Deborah Tuttle & John Piper in memory of "Rosie" CH Scrimshaw's Rose of Northstar and "Rebel" GCH Nautique's Stars And Bars AOM AWD

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Sigrid & Neil Bundy in memory of Aarion's Bela Marina UD RE NA NAJ

Janice Butler in memory of Lisa Humke

Janice Butler in memory of my sweet Angus – you will always be my heart dog!

Paul & Ann Byrum in thanks to Meg DeFore, Beth Mercier and Torrid Zone.

April Carter in loving memory of our sweet Gabbie and adorable Cinco who crossed the rainbow bridge too soon.

Annette & Scott Castiglione (Bella)

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Pat Devries in honor of the PWD PSG

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Michael & Jennifer Greene – Thank you to Windruff Kennels for Nautica

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Angela Harding in memory of Bev Rafferty

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Silke Hirtz-Schmidt in honor of the PWD PSG

Sandra Holden for all PWDs who have crossed over the Rainbow Bridge in 2016

Otto & Cynthia Kuehne in memory of Kolbe 12/05/2006 – 07/01/2014

Randy & Karen Latham

Sarah Leatherman in memory of Tess, beloved companion of Bea and Gordon Jennings and Buddy

Susan Lefebvre in memory of Aquarelle's Zoe Laporte

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Pam Marshall in thanks to Sandy & Ken Melson for hosting the 8th Vista do Lago's Water Camp, and trainer, Judy Murray

Theresa McConnell

Eileen Minder

Cindy Miner in thanks to Benita Bottom and Louann Tracy – best training partners ever!

Heather Morrissey

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United Sunshine State PWD Club in memory of one of its founding members, Gary Stern

United Sunshine State PWD Club in memory of Mara Hertzberg's son, Tyler

William Varr & Victoria Morro

Boatswain \$100-\$249 (continued)

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CH Galaxy's Calabresella CD RN
Susan & Don Wells in memory of Frank Carter
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Paul Zoschke & Marcie Wallace in celebration of
Cheers 16th Birthday!

Sailor \$50-\$99

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Eliot Brown & Mary Sama-Brown
Mary Jo Burgess
Ann Camp in honor of the PWD PSG
Linda Campbell in honor of the PWD PSG
Sherry & Dan Cassidy-Porter in memory of CH
Driftwood's Magic Kiss O Love "Smooch." You
will forever be missed.
Julie Conger in honor of the PWD PSG
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Sue & Bill Evans in memory of Meia, Sculler &
Fred
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Barbara Floch in honor of the PWD PSG
Fred & Susan Forman in honor of the PWD PSG
Dr. Richard A. Frankel
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Angela Harding in honor of the PWD PSG
Katrina Jackson in honor of the PWD PSG
Katrina Jackson in honor of the PWD PSG
Dana Johnston in honor of Nemo & Neptune
(Mariner)
Jere & Connie Joiner in memory of PWD Caesar,
hemangiosarcoma, age 8
Peter & Gerd Jordano wishing Happy Birthday to
their PWD "Mama Mia" - 14 years old on
12/25/2016
Carole & Ed Kainen

Elizabeth Kappes
Ann & Alan Koehler in memory of Lacey
Michael & Dona Lee
Warren & Sandra Lloyd in honor of the PWD
PSG
Kathy Maguire in honor of the PWD PSG
John McCredie
Jane & Chris McSweeney in memory of their
sweet, sassy Katie
Michael Mobley in honor of the PWD PSG
Kathy Krekow Moe
Louise Mowbray
Michael Otchet
Joyce Polak in honor of the PWD PSG
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Julie Rust in honor of the PWD PSG
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"Schooner" Legado Guardascostas Schooner
Rio Salgado PWD Club in memory of
"Buccaneer" Amarinhar Hearty Buccaneer AX
OAJ OF OAP OJP AWD JWDC EAC EJC ECC
TN-N TG-N WV-N HP-N Novice Versatility Award
Rio Salgado PWD Club in memory of "Sparkle"
Aqua Viva's Sparkling Diamante
Rio Salgado PWD Club in memory of Caleb
Donna Sack
Donna Sack in memory of a much loved "Tess"
Donna Sack in honor of the PWD PSG
Sandra Saybolt in honor of the PWD PSG
Elsa Sell in honor of the PWD PSG
Linda Shultz
Phyllis Shurzinski in memory of Seabreeze Kiss
My Grits a/k/a Alice Marie
Elaine & Mark Suter in honor of the PWD PSG
Dr. Cindy Tanenbaum & Ezra Simon
Janet Warnsdorfer in loving memory of "Maya"
Galaxy's Sky's the Limit

Janet Warnsdorfer in loving memory of "Sophie"
Galaxy's Shoot the Moon
Janet Warnsdorfer in loving memory of "Sailor"
Galaxy's Luke Skywalker
Gary & Sandi Willis in memory of Bella & Lori
Sian Wilstrup in honor of the PWD PSG
Elana Winsberg in memory of Marisol
Theresa Zorad in honor of the PWD PSG

Deck up to \$49

Cheryl Blancett in memory of Dawn Smith
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Katrina Jackson