



# THE WINN FELINE FOUNDATION

For the Health and Well-Being of All Cats

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## **2007 FELINE HEALTH GRANT AWARDS**

*Eight studies funded for a total of \$127,544*

The Winn Feline Foundation is pleased to receive proposal from veterinary researchers around the world who are interested in improving feline health. Out of 40 proposals for 2007, our team of outstanding veterinary consultants helped the Foundation select the best studies for funding. We look forward to seeing the results of these studies and being able to share them with the veterinary community as well as cat owners and pedigreed cat breeders.

### **CONTINUATION OF PREVIOUSLY FUNDED STUDIES**

*Targeted gene mapping in gaps of the feline-human comparative map*

William J. Murphy, PhD; Texas A&M University; \$14,585

The male-specific region of the Y chromosome is one of the most divergent regions in the mammalian genome among species. Studies of the human and mouse Y chromosomes, and comparison to other mammals, have shown that Y chromosomes have acquired a unique repertoire of testis-specific genes that enhance male reproductive function. Several of these genes are associated with infertility. Though there is an extensive descriptive background on reproductive physiology and spermatogenic defects in felines, nothing is known about the genes involved in these processes. Furthermore, the existing shotgun-based feline whole genome is based on a female genomic DNA template, and there are no plans to sequence the Y chromosome of the domestic cat. To fill this void, Dr. Murphy will extend his initial studies of the cat Y chromosome by sequencing from a direct cDNA selection library enriched for Y chromosome transcripts. This procedure will isolate novel transcribed cat Y chromosome genes, which will be mapped in the cat genome. The expression patterns of each gene will be determined in a range of tissues to discern which genes have testis-specific expression patterns, and may therefore be good candidates for abnormal sperm morphology. The identification of novel Y chromosome genes involved in feline spermatogenesis will provide targets for future analysis in both domestic cat lines and exotic felid species, of which many have high frequencies of sperm abnormalities. This study will be critical for filling the final major 'gap' in the cat genome project.

### **BREED-FUNDED STUDIES**

*Molecular characterization of feline COX-2 and expression in mammary cancer*

Monique Doré, DVM, MSc, PhD, DACVP; University of Montreal; \$15,000

Mammary tumors are among the most common neoplasms in cats. Evidence indicates that cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the biosynthesis of prostaglandins, plays a role in mammary tumorigenesis in humans and dogs. However, currently available information about the expression of COX-2 in feline mammary cancer

is very limited and conflicting. Moreover, the primary structure of feline COX-2 has not yet been characterized. Therefore, the objectives of this proposal are to characterize the structure of feline COX-2 and to study its expression in mammary adenocarcinomas. Our hypothesis is that feline mammary adenocarcinomas overexpress COX-2. More specifically, this project will address two objectives: 1) to characterize the primary structure of feline COX-2, and 2) to determine the expression of COX-2 in feline mammary cancer. Information gained from these studies will help develop tools that will be used in future investigations aimed at exploring the regulation of COX-2 in feline normal and cancerous cells. It is expected that a better understanding of the fundamental mechanisms involved in prostaglandin synthesis in feline mammary cancer will lead to innovative strategies for the prevention and treatment of this widespread and deadly disease in cats.

**[This study was partially funded by the efforts of the Siamese Breed Council and many other interested breeders.]**

*Molecular evaluation of the feline myosin heavy chain gene in Ragdoll, Norwegian Forest and Sphynx cats with familial hypertrophic cardiomyopathy*

Kathryn M. Meurs, DVM, PhD, DACVIM; Washington State University; \$31,550

Feline hypertrophic cardiomyopathy (HCM) is the most common cause of heart disease in the adult cat. Affected cats are at risk of sudden death, heart failure or an arterial thrombus. Feline HCM is familial in the Maine Coon (MC), Ragdoll, Sphynx and Norwegian Forest (NWF) cat. In human beings, the disease is associated with a mutation in one of several sarcomeric genes, and the myosin binding protein C (MYBPC) and myosin heavy chain genes are reported most commonly. We previously demonstrated that HCM is associated with a mutation in the MYBC gene in the MC cat. We have collected pedigrees and DNA samples from Ragdolls, NWF and Sphynx cats with familial HCM. The MC cat mutation was not present in any of these breeds. Further evaluation of exons of the MYBPC and two other sarcomeric genes, troponin I and myosin light chain 2 in the Ragdoll and NWF cat have not identified a causative mutation. We hypothesize that a mutation in the myosin heavy chain gene is associated with the disease in the Ragdoll, NWF or Sphynx cat. Although it is unlikely to be the same mutation in all three breeds, it is very possible that all three may have mutations at different locations within this gene. The objective of this study is to identify a causative mutation in the coding or splice site regions of the myosin heavy chain gene in affected cats from these three breeds.

**[This study was largely funded by the efforts of Ragdoll, Norwegian Forest Cat and Sphynx breeders. This study was also supported by the Ricky Fund, a fund for the study of feline hypertrophic cardiomyopathy established by Steve Dale in memory of his cat, Ricky.]**

## NEW STUDIES

### *Prevalence and risk factors for venereal Tritrichomonas foetus infection*

Jody L. Gookin, DVM, PhD; North Carolina State University; \$12,465

Feline *Tritrichomonas foetus* (TF) infection is a significant clinical problem. The infection is prevalent among pedigreed cats where it causes chronic large bowel diarrhea. Ronidazole, identified as effective in clearing infection, is expensive, potentially toxic, and may fail to cure some cats, making eradication of TF from a cattery extremely difficult. In cattle, TF is a venereal disease, where treatment failure is common and attributed to seclusion of the infection in the bulls' genitalia. We have observed that treatment failure in cats appears to be more common in males and were able to amplify TF DNA from the epididymis of a male cat with intestinal infection. This study will determine if the reproductive tract serves as a reservoir for TF in populations of cats at high risk for intestinal infection. We will examine the reproductive tract of 100 cats undergoing spay or neuter by offering about 2,500 cattery owners free fecal TF PCR testing for any cat from which we receive both a fecal and reproductive tract specimen. Specimens will be tested for TF DNA by single-tube nested PCR. Housekeeping PCR reactions for bacterial 16S and GAPDH will be used to evaluate for fecal contamination of reproductive specimens (false+) or PCR inhibitors (false-). TF organisms will be identified in PCR+ samples by immunostaining tissues with TF-specific antibodies. Venereal involvement in feline TF may explain examples of treatment failure and high prevalence of disease in breeding programs and has significant implications for the design of effective treatment and prevention strategies.

### *Evidence of effective drug delivery using transdermal gel delivery systems in cats*

Dawn Boothe, DVM, PhD, DACVIM, DACVCP; Auburn University, \$14,990

Multiple studies in cats have demonstrated that drugs delivered via topically applied pluronic lecithin (PLO) gels do not predictably reach the blood stream. Yet, PLO gels continue to be promoted and compounded by pharmacists and prescribed by practitioners. Further, practitioners anecdotally report clinical response with their use. The discrepancy between experimental results and clinical use may reflect study duration, which generally has been a single dose. Multiple dosing may be necessary before drug penetration is effective and response can occur. This study proposes to document the presence of drugs administered as PLO gels in spontaneously ill cats. Three drugs will be studied: methimazole, metronidazole, and prednisolone/prednisone. Accuracy of compounded products and stability (potency) throughout the dosing period will be determined for each drug administered to 25 cats as a PLO gel and 25 matching (control) cats receiving the drug orally. Blood levels (peak and trough) will be monitored on day two, and intermittently for a maximum of 4 times for up to 3 months. Evidence of clinical response will be based on client and veterinary assessment. Endpoints will be compared between oral and PLO routes. Endpoints will include, for each cat, the magnitude of plasma drug concentrations, time to peak concentration, time to therapeutic concentration, and therapeutic response (yes or no). For each product, percent accuracy of compounded product and duration of potency will be determined such that an expiration date can be offered.

*Characterization of feline immune responses to recombinant DNA vaccines against avian H5N1 influenza virus*

Elizabeth W. Uhl, DVM, PhD, DACVP; University of Georgia; \$15,000

The discovery that domestic cats have not only been naturally and fatally infected with the H5N1 avian influenza virus, but have transmitted it to other cats, raises concerns about their potential role in a global pandemic. In the event of a pandemic, vaccine manufacturers do not have the resources to produce inactivated or modified live viral vaccines for both humans and cats, therefore other types of vaccines need to be developed to protect pets. A recombinant DNA vaccine expressing antigens of H5N1 influenza is safer, easier and less expensive to make since DNA is used as the immunogen rather than whole virus. The goal of this project is to develop a recombinant DNA vaccine that induces potentially protective immune responses in cats. To achieve this goal, we have assembled a collaborative team with the expertise and reagents to make the vaccines, assess feline immune responses, and insure the proper and humane handling of cats. Neutralizing antibody and cell-mediated immune responses generated in cats vaccinated with recombinant DNA vaccines encoding the HA or nucleoprotein (NP) antigens of H5N1 avian influenza will be assessed. Experimental groups will consist of cats receiving: 1) plasmid vector alone; 2) plasmid vaccine with cDNA encoding the HA of an H5N1 influenza virus; 3) a vector encoding influenza A NP cDNA; and 4) H5N1 inactivated influenza vaccine. To assess the serum antibody response, anti-HA and NP ELISA and hemagglutination inhibition (HI) assays will be performed. Cell proliferation and interferon- $\gamma$  assays will be used to assess cell mediated immune responses.

*Detection of anti-erythrocyte antibodies in cats with anemia*

Kristy L. Dowers, DVM, MS, DACVIM; Colorado State University; \$8,954

Primary immune-mediated hemolytic anemia (IMHA) is thought to be uncommon in cats; secondary IMHA, induced by neoplasia or infectious agents, is considered common. Neither mechanism can be detected reliably using standard Coombs' testing to identify anti-erythrocyte antibodies. Recent studies suggest that primary IMHA may be more common in cats than previous thought. The purpose of this prospective study is to identify a subset of cats with primary IMHA and to develop more sensitive tests for detecting autoantibodies in cats with either primary or secondary IMHA. Whole blood and serum samples from 7 healthy client-owned cats and 43 client-owned cats presenting for acute anemia will be analyzed using polymerase-chain reaction assays for infectious agents known or suspected to cause secondary IMHA (*Mycoplasma haemofelis*, 'Candidatus M. haemominutum,' feline leukemia virus (FeLV), *Ehrlichia canis*-like organisms, *Anaplasma phagocytophilum*, *Cytauxzoon felis* and *Bartonella* spp) and evaluated for anti-erythrocyte antibodies using the Coombs' test. Flow cytometry and an ELISA assay, two previously untested techniques in cats, will be validated and compared to the Coombs' test. The recognition of primary IMHA in cats and the use of more sensitive techniques to characterize feline IMHA may lead to new diagnostic and therapeutic approaches to the disease.

*Mesenchymal stem cell transfer for treatment of chronic renal disease in cats*

Steven Dow, DVM, PhD; Colorado State University; \$15,000

Chronic renal disease remains the leading cause of death in cats and there are still no effective treatment options short of renal transplantation. Stem cell therapy using bone marrow derived stem cells has recently been shown to improve renal function in rodent models of renal failure. Stem cells can augment renal function by trans-differentiation into functional renal tubule cells and by local production of growth factors that improve the function of existing tubule cells. In particular, mesenchymal stem cell (MSC) transfer has been shown to promote renal tubule development and suppress development of renal fibrosis. Our lab has developed the techniques necessary to establish mesenchymal cell cultures from cat bone marrow. Therefore, we propose to conduct a pilot study to evaluate the safety and potential efficacy of MSC transfer in 9 cats with naturally-occurring chronic renal disease. Autologous MSC will be generated from autologous bone marrow samples, then expanded *in vitro* prior to transfer. One MSC sample will be injected into multiple sites on both kidneys of each cat, using ultrasound guidance. Three groups of cats ( $n=3$  cats per group) will be injected with increasing numbers of MSC. The effects of MSC transfer on renal function, including GFR, will be assessed over a 3 month period. Safety will also be assessed by physical examination, urinalysis and blood work. This study will provide an important proof-of-principle assessment of this promising new approach, which could be readily applied clinically to many cats with chronic renal disease.

**For more information, contact:**

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