

## **Buttons Fund for Feline Chronic Kidney Disease Research Annual Report 2022**

The Buttons Fund for Feline Chronic Kidney Disease Research supports cutting-edge research in chronic kidney disease in cats as well as other feline-related research. Under the direction of Dr. Jessica Quimby, DVM, PhD, DACVIM (Internal Medicine), this research includes projects designed to understand the mechanisms of renal aging and disease, assess the efficacy of medications to improve the outcome and quality of life for chronic kidney disease cats, as well as investigations into novel treatment strategies, which are then tested via a rigorous clinical trials program.

### **Current Fund Vision**

- One of the major aims of the fund is to support clinical studies for CKD cats, typically ranging from \$10,000-30,000 per study.
- Another major fund aim is to support veterinary students' involvement in feline research. For several years Buttons Fund has supported students to participate in the Summer Scholars Program (\$5000/student).
- A second educational aim is to mentor young doctors (interns and residents) in their involvement in feline clinical trials. Currently six young doctors are participating in projects supported by Buttons Fund. Support of these research studies promotes interest in feline clinical trials and helps residents meet the publication requirement for board certification in small animal internal medicine.
- These opportunities enhance understanding of interacting with cats in a clinical trials setting, and the importance of advancing feline medicine.
- Our future vision is also to continue the feline research intern experience, as well as attracting Master's and PhD student candidates. These positions range from \$50,000-70,000 a year in salary support and can be fully or partially supported by the fund.

### **Studies Supported By Buttons Fund in 2022**

#### The following studies were completed:

- *Evaluation of Quality of Life in Cats with Chronic Kidney Disease*
  - The perception of a cat's quality of life is multifactorial and we know that health has a significant impact. The purpose of this study is to combine medical information from the patient with information collected from a quality of life survey completed by caretakers of cats with CKD in order to identify factors influencing the perception of quality of life. This project utilizes a health related QOL survey previously developed by Jacky Reid at Glasgow University and now available as a commercial assessment tool (Button's Fund has assisted by paying the fees to access this assessment tool). Caretakers were asked to complete the online survey at the time of a veterinary visit in which labwork is

- performed. This study demonstrated that several factors, including severity of disease, anemia, constipation and muscle condition were associated with a decrease QOL score.
- Dr. Sarah Lorbach is the internal medicine resident who has been working on this project. She was Dr. Quimby's research intern prior to starting her residency.
  - *Abstract:* Lorbach S, Quimby JM, Nijveldt E, Paschall R, Reid J. Evaluation of health-related quality of life in cats with chronic kidney disease. American College of Veterinary Internal Medicine Forum 2022.
  - *Publication:* manuscript in progress
- *The impact of fecal identification markers on the feline microbiome*
    - Collection of fecal samples from cats in order to study the microbiome can be challenging. In order to identify which cat in a multicat household is producing which feces, non-toxic, non-irritating markers such as glitter or crayon can be fed. However it is not known if these markers will affect the microbiome. The purpose of this study was to assess the effect on the microbiome. Unfortunately it was found that these markers do affect the microbiome and should not be used for this type of research.
    - Dr. Ali Wood, Dr. Hannah Klein and Dr. Nora Jean Nealon in collaboration with Dr. Jenessa Winston, an internal medicine specialist who studies the microbiome, were our collaborators on this study which was Dr. Wood's master's thesis.
    - *Abstract:* Wood A, Nealon NJ, Klein H, Rudinsky A, Salerno M, Quimby JM, Parker V, Howard J, Winston J. The impact of fecal identification markers on the feline microbiome. American College of Veterinary Internal Medicine Forum 2022.
    - *Publication:* this manuscript has been submitted for publication to Frontiers in Vet Med.
  - *Untargeted metabolomics in young adult cats, senior cats and cats with CKD*
    - In order to better understand the association between gut dysbiosis and increased production of gut-derived uremic toxins, we need to better understand the functionality of the intestinal microbiome by integrating characterization of the intestinal microbiome with the evaluation of the metabolites produced by the gut microbiome. This project involves performing untargeted metabolomics on a set of serum samples from CKD that we previously examined the microbiome on. This allows us to evaluate the functional consequence of the altered fecal microbiome. This project is part of continued collaborations with Dr. Stacie Summers at Oregon State University.
    - *Abstract:* Summers S, Quimby JM, Winston J. Untargeted metabolomic profiling of serum from cats with chronic kidney disease. American College of Veterinary Internal Medicine Forum 2022.
    - *Publication:* manuscript in progress

- *To sample or not to sample: capturing feline fecal microbiome changes with high-frequency sample collection*
  - In order to study the feline microbiome, it is necessary to better understand how best to collect representative samples. This study looks at sample frequency to determine how many samples are necessary for an accurate assessment. It was found that more than one sample is ideal to accurately describe a cat's microbiome.
  - Dr. Nora Jean Nealon who is Dr. Jenessa Winston's post-doctoral research fellow and has significant microbiome experience spearheaded this project.
  - *Abstract:* Nealon NJ, Klein H, Salerno M, Rudinsky A, Quimby JM, Parker V, Howard J, Winston J. To sample or not to sample: capturing feline fecal microbiome changes with high-frequency sample collection. American College of Veterinary Internal Medicine Forum 2022.
  - *Publication:* manuscript in progress
  
- *Expression of CD31 in feline kidneys to characterize peritubular capillary density in CKD*
  - Feline CKD is characterized by tubulointerstitial inflammation, tubular loss and fibrosis. Hypoxia is a key driver of fibrosis and its pathophysiology is associated with capillary rarefaction (reduction in vascular density) in humans. The goal of this study was to determine if similar vascular changes occur in cats with CKD. This study found that some cats with CKD did have very small percentage of capillary area remaining in their kidney.
  - Dr. Rene Paschall participated in the Summer Scholars program as a veterinary student supported by Buttons Fund in 2018 to initiate this project. Dr. Paschall has completed Dr. Quimby's research internship, a rotating internship and is currently an internal medicine resident.
  - *Abstract:* Paschall R, Quimby JM, Cianciolo RE, Mclelland SM, Lunn KF, Elliott J. Assessment of capillary rarefaction in cats with and without CKD. Presented at American College of Veterinary Internal Medicine Forum 2021. Poster Presentation.
  - *Publication:* This paper was submitted to J Vet Intern Med and is currently under review.
  
- *Validation of Ghrelin Sample Storage and Stability in Healthy Cats*
  - Ghrelin is often called the "hunger hormone". Unfortunately, ghrelin requires very particular handling and storage to maintain its chemical structure. The purpose of this study is to assess differences in blood sample storage protocols and whether prolonged storage affects the concentration of this hormone within blood samples. This study is to prepare to evaluate differences in appetite regulation between healthy cats and those with chronic kidney disease. It was found that acidifying the samples does increased their stability during storage. Excitingly this pilot data allowed us to successfully apply

- for a grant from EveryCat Health Foundation (W21-042 Hormonal regulation of appetite in cats with and without CKD) to further this work!
- Dr. Sarah Lorbach is an internal medicine resident. She was Dr. Quimby's research intern prior to starting her residency.
  - *Abstract:* Lorbach SK, Quimby JM, Brusach KK, Kinsella H, Toribio R. Ghrelin sample storage and stability in healthy cats. Presented at American College of Veterinary Internal Medicine Forum 2021. Poster Presentation.
  - *Publication:* This paper will be resubmitted for publication by the end of December.
- *Histologic assessment of the aging feline kidney in cats without kidney disease.*
    - In humans, histopathologic changes occur in the kidney even before clinical kidney disease is evident. The goal of this study was to describe the pathology present in the kidneys of cats over a wide age range who had no clinical indication of CKD. It was found that similar to humans, renal aging in cats without CKD is characterized by increasing glomerulosclerosis, tubular atrophy, interstitial inflammation, fibrosis and frequency of fibrointimal hyperplasia. Tubular and interstitial lipid also increase with age, and this lesion may be unique to domestic cats.
    - Dr. Shannon McLeland is a cat-loving board-certified anatomic pathologist who collaborates with our research group from her home in Minnesota, having previously completed a PhD on feline CKD at Colorado State University under the direction of Dr. Quimby.
    - *Abstract:* Mcleland SM, Quimby JM, Cianciolo RE, Lunn KF, Lulich JP, Herndon AK, Zajic LB. Histologic assessment of the aging feline kidney in cats without kidney disease. Presented at American College of Veterinary Internal Medicine Forum 2019. Poster Presentation.
    - *Publication:* This paper was recently accepted for publication in J Fel Med Surg.

The following studies are underway:

- *Assessment of HIF-1 and HIF-2 expression in feline kidneys*
  - Hypoxia is a major factor in the development of fibrosis in the kidney and HIF is a master gene in the regulation of hypoxia. Characterizing the expression of HIF in the feline kidney of cats with and without CKD will improve our understanding of hypoxia in feline CKD.
  - Dr. Rene Paschall participated in the Summer Scholars program as a veterinary student supported by Buttons Fund in 2018 to initiate this project. Dr. Paschall has completed Dr. Quimby's research internship, a rotating internship and is currently an internal medicine resident.

- *Fecal bile acids in cats with CKD*
  - Fecal bile acids have been little studied in cats, and may be another biomarker of gut dysbiosis associated with CKD. This project is another collaboration with Dr. Stacie Summers and characterizes the composition of bile acids in cats with CKD as compared to healthy geriatric cats. Sample collection and initial sample analysis is complete and the manuscript is being prepared for publication. Some of the data were presented as an abstract at ACVIM Forum 2020. Secondary data analyses are currently underway (delayed due to COVID) under the direction of Dr. Janessa Winston, an internist who specializes in microbiome and bile acid biology.
  
- *Assessment of Erythrocyte Fragility in Cats with CKD*
  - Anemia is one of the major complications of CKD, affected 30-65% of feline CKD patients. Although the main contributor to this process is thought to be a decrease in erythropoietin and thus decreased bone marrow stimulation, increased red blood cell (RBC) fragility has also been posed as a mechanism that shortens RBC life span. The aim of this study was to explore the effect of CKD and uremic toxins on the fragility of feline RBC using an osmotic fragility assay.
  - Muning Wang, a veterinary student, participated in the 2020 Summer Scholars supported by Buttons Fund to initiate this project. The data collection for this study is completed and analysis is underway.
  
- *Biometrics of Feline Chronic Kidney Disease*
  - We have an IACUC protocol for collection of routine labwork (chemistry, CBC, UA, T4, blood pressure) in CKD cats and normal cats to allow us to obtain samples as needed for biobanking and to support the clinicopathologic needs of such studies as the Quality of Life Survey. This also allows owners who may not be able to afford full labwork for their cat to participate in screening for clinical trials.

The following initiatives are starting:

- *Feline Renal Biopsy Initiative*
  - We plan to offer renal biopsy/SDS PAGE analysis by our lab (the International Veterinary Renal Biopsy Service; <https://vet.osu.edu/vmc/international-veterinary-renal-pathology-service-ivrps>) for free for a limited period of time (potentially the next three years) to encourage increased submission of feline samples, including necropsy samples (paired kidney and urine). Although the renal biopsy service is quite busy we routinely get significantly fewer feline samples. This initiative will allow us to obtain additional materials via kidney donation for biobanking and provide materials for investigation of biomarkers of disease. Analysis by the IVRPS included special stains, EM and IF analysis

by a board-certified nephropathologist as well as SDS PAGE urine evaluation and is typically ~\$700 per sample. This project has been delayed because the IVRPS has recently moved to Texas A&M, but preparations will resume early 2023.

### Dissemination

Recent publications supported by Button's Fund:

Summer S, Quimby JM, Blake A, Keys D, Steiner J, Suchodolski J. Serum and fecal amino acid profiles in cats with chronic kidney disease. *Vet Sci.* 2022; 9: 84. doi: 10.3390/vetsci9020084.

Dr. Quimby is an international speaker and key opinion leader focusing on feline medicine and chronic kidney disease in particular. She seeks to increase awareness of research needs for chronic kidney disease as well as provide updates on recent studies to disseminate information obtained by the program and speaks at several national and international conferences a year.

### Awards

Dr. Quimby was elected to the International Renal Interest Society (IRIS) Board. The mission of IRIS is to help veterinary practitioners better diagnose, understand and treat renal disease. (<http://iris-kidney.com/>)