# RESEARCH PROGRESS REPORT SUMMARY

**Grant 02152:** Translation of MicroRNA into an Early Diagnostic Test for Chronic Kidney Disease

**Principal Investigator:** Mary Nabity, DVM, PhD

**Research Institution:** Texas A&M AgriLife Research

**Grant Amount:** $26,988.00

**Start Date:** 1/1/2015 **End Date:** 12/31/2018

**Progress Report:** Mid-Year 4

**Report Due:** 6/30/2018 **Report Received:** 6/22/2018

---

*(The content of this report is not confidential and may be used in communications with your organization.)*

**Original Project Description:**

Chronic kidney disease (CKD) is a significant cause of illness and death in dogs and is often due to glomerular diseases. Dogs with glomerular disease often have poor outcomes with standard therapy, and specific treatment recommendations are difficult without performing a kidney biopsy to determine the type of glomerular disease present, since treatment and outcome among these diseases differs substantially. Even then, we lack an understanding of the mechanisms driving these diseases, limiting our ability to optimally treat these dogs. Therefore, tests to non-invasively diagnosis the type of glomerular disease would help veterinarians more appropriately treat these patients and provide insight into the mechanisms that cause the diseases. This could lead to better therapies that slow disease progression and improve quality and length of life in dogs with CKD.

One area of emerging importance in CKD is the role of microRNAs ( miRNAs) in disease pathogenesis and progression. miRNAs are small molecules that can regulate gene expression by up or down regulation of messenger RNA transcripts and proteins in target tissues. Many studies have found that increases or decreases in miRNAs can serve as biomarkers of diseases, including human CKD. They also contribute to the development of diseases. The goal of Dr. Nabity's study is to identify miRNAs in serum and urine of dogs that are specific for the three major causes of glomerular disease in this species. They also aim to identify miRNAs associated with disease progression for each of these diseases. Successful completion of these goals will support the translation of miRNAs into diagnostic tests and viable targets for future drug development.

**Publications:**

We have several manuscripts that are in varying stages of preparation. Two that are nearly completed and are anticipated to be submitted for review this summer are:

1) Technical Aspects in Isolating and Profiling Circulating and Urinary MicroRNAs in Domestic Animals This manuscript is a review that will serve as a resource for veterinarians interested in miRNA research.

2) Comparison of RNA isolation and library preparation methods in canine biofluids for small RNA sequencing

In addition, we are working on a manuscript detailing the findings of the small RNA--seq results ("MiRNA profiling of serum and urine in dogs with glomerular diseases"). We may wait toinclude the qRT--PCR results, or we might submit the qRT--PCR study as a separate manuscript.

**Presentations:**

Dr. Candice Chu, a PhD student in my laboratory, presented an abstract entitled: "Comparison of methods for preparation of biofluids in dogs for small RNA sequencing" (oral presentation) at the 2017 ACVP/ASVCP Concurrent Annual Meeting November 4--8 in Vancouver, BC. She received the 2017 ASVCP Young Investigator Award for this presentation. She also presented this talk at our TAMU--CVM graduate student symposium and won the 'People's Choice Award' for her presentation.

**Report to Grant Sponsor from Investigator:**

MicroRNAs (miRNAs) are small, non--coding RNAs that can alter gene expression, and they can serve both as biomarkers of disease and instigators of disease progression. The goal of this project is to identify miRNAs as new biomarkers in the serum and urine of dogs with kidney disease due to primary glomerular diseases. In particular, our goal is to identify miRNAs that can non--invasively (i.e., with a blood or urine sample) distinguish among the 3 most common glomerular kidney diseases in dogs. Currently, such non--invasive identification of these diseases is not possible;; yet, their treatment and prognosis vary considerably.

The first objective of this project was to identify and quantify all miRNAs present in the serum and urine from dogs with each of the 3 glomerular diseases, both early and late in the disease process. This was performed using a technique called small RNA--sequencing. We recently received the sequencing results, and data analysis revealed a number of miRNAs that were either increased or decreased in the urine and serum of dogs with kidney disease compared with healthy dogs. Additionally, several miRNAs were increased in the urine of dogs with late disease versus early disease. Our most exciting finding is that there were 3 miRNAs

that appear to differentiate among the 3 most common categories of glomerular disease in dogs. Our next step (Objective 2) is to verify these findings with a faster, less expensive technique (PCR) using a larger number of samples. If verified, these findings would provide a way for veterinarians to determine whether immunosuppressive therapy is warranted in a dog with proteinuric kidney disease without obtaining a kidney biopsy. While non--invasive tests are unlikely to ever provide as much information as a kidney biopsy, being able to accurately determine appropriate therapy using a urine sample would benefit those patients that are poor candidates for having a kidney biopsy or for owners that can't afford it. We anticipate completion of Objective 2 by the end of 2018.

While we obtained the sequencing data much later than we originally intended, we have gained substantial experience with methods for RNA evaluation in urine and serum. For instance, we gained experience with 10 different urine and serum RNA isolation methods. Furthermore, there is currently no standardly accepted method for RNA isolation and preparation for small RNA--sequencing from urine and serum samples. Therefore, we performed a method comparison study. The experience we gained with small RNA sequencing through this method comparison study not only helped ensure optimal use of our limited study samples, it also helped us improve upon our original study design and was imperative to ensuring sequencing success. In fact, one method that initially appeared promising failed to produce any results and would have caused a significant setback had we used this method for our study samples.

Thus far, this project has provided research experience to 2 graduate students and served as a significant portion of a PhD thesis for one of these students who recently defended. Another graduate student will help with the project going forward to provide additional support, which should help ensure timely completion of Objective 2. The graduate student who recently defended her dissertation has won an award at both a local and a national conference for her presentations of data generated during this study.