The GI Lab
VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY
Promoting Gastrointestinal Health in Companion Animals
Newsletter - Summer 2022
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What's Inside This Issue:

News from the Gastrointestinal Laboratory

Another year has passed, and once again we have made it through a strange, yet very exciting period. I am very happy to report that our team is growing. Since my last column, three new faculty members have joined the GI Lab and a fourth, who will work closely with us, has recently joined our Department:

Dr. Emily Gould (pictured to the left) joined our team of internists on September 1, 2021. Emily received her veterinary degree from the University of California at Davis in 2012 and pursued a combined residency/PhD program at the University of Tennessee. When her mentor Dr. Katie Tolbert transferred to the GI Lab, Emily also moved to Texas A&M in order to complete her PhD program here. Emily's PhD project focused on the effects of acid suppressants on mast cells and mast cell tumors. We feel extremely fortunate that Emily has decided to stay in Texas after completion of her PhD. For the past two years she has already been taking your consultations, but now you will have the opportunity to talk with her on a more regular basis.

Starting at the beginning of 2022, Dr. Paula Giaretta (pictured to the right) joined us, having previously worked at the Federal University of Minas Gerais in Belo Horizonte in Brazil. Paula received her veterinary degree from the Federal University of Santa Maria, Brazil, in 2012 and completed a combined residency/PhD program in anatomic pathology here at Texas A&M University in 2019 before returning to Brazil. We feel very fortunate that she has chosen to spend the next part of her professional career with us. While Paula has a partial appointment in the Department of Veterinary Pathology, her main appointment is in the GI Lab where she is leading our efforts in gastrointestinal histopathology, as well as immunohistochemistry and other advanced histopathologic tools.

Also, starting in early 2022, Dr. João Cavasin (pictured to the left) joined our team from Cornell University. João graduated from the Federal University of Parana, Brazil, in 2017 and just completed a residency in anatomic pathology at Cornell University. Similarly to Dr. Giaretta, Dr. Cavasin also has a partial appointment in the Department of Veterinary Pathology, but his main appointment is in the GI Lab where his focus is on hepatic histopathology. Dr. Cavasin has worked with Dr. Sharon Center, Dr. Sean McDonough, and others at Cornell University, and we are very fortunate that he has decided to join our team.

Finally, Dr. Kate Aicher (pictured to the right) has come back to Texas A&M University. You may recall Kate from her time as the lab manager of our service lab many years ago. After attending veterinary school at Texas A&M, she pursued a residency in small animal internal medicine at North Carolina State University and spent several years in private practice as a specialist and the chief medical officer of a specialty referral hospital. As a faculty member at Texas A&M University, Kate plays a major role in teaching veterinary students at our VERO West Texas Campus. However, she also works closely with the GI Lab. She will be answering your questions on complex gastrointestinal cases and is involved in some of our research activities.

At the GI Lab, we work to continually improve our facilities in order to make sure we are always ready to serve our missions of service, research, and outreach. We just finished an expansion of

(continued on next page)

our Metabolomics lab and have updated our small molecule analysis capacity. We now have two gas chromatography/mass spectrometry (GC/MS) systems that we use for the measurement of bile acids, fatty acids, N-methylhistamine, and methylmalonic acid, amongst others. We also have an automated high- performance liquid chromatography system to simultaneously measure more than 40 amino acids in serum samples. Finally, we recently purchased a brand new liquid chromatography with tandem mass spectrometry instrument, which will allow us to simplify many of our current GC/MS-based assays.

We continue to be excited about the future – there is a lot more research to be done towards the development of new diagnostic tests that will help us to better and more easily diagnose our patients as well as to offer better treatments to manage their conditions. We would not be able to do this work without your help – thank you for your continued patronage! (Joerg Steiner)

Canine Alpha-1 Proteinase Inhibitor Test Available Again

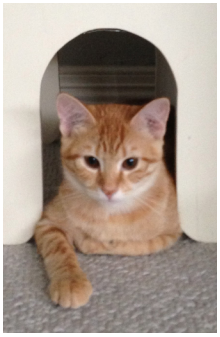
We are pleased to announce that we once again can offer the fecal alpha-1 proteinase inhibitor (α_1PI) assay as a marker for gastrointestinal protein loss in dogs. Because canine α_1PI is more resistant to degradation than other proteins, such as albumin, it serves as a specific marker to detect excessive gastrointestinal protein loss as can occur with protein-losing enteropathies (PLE). A variety of diseases, including lymphangiectasia or chronic inflammatory enteropathy, can cause PLE.

A mean three-day canine α_1PI concentration of ≥ 13.9 µg/g feces or a canine α_1PI of one individual sample of ≥ 21.0 µg/g feces is considered abnormal.

Naturally voided fecal samples (about 1 gram per sample) should be collected immediately following defecation on three consecutive days. Special pre-weighed fecal tubes must be used and are available from the Gastrointestinal Laboratory. To order fecal tubes, please email us at gilab@cvm.tamu.edu.

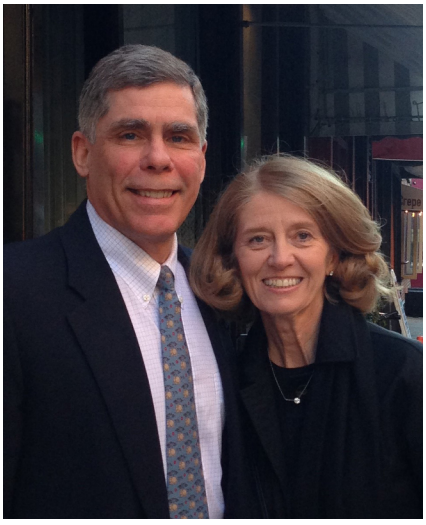
Samples should be frozen until submission to our lab and should be shipped on ice by overnight courier. (Katie Tolbert)

Dr. Jonathan Lidbury named the Rob and Roxann Bilger Chair in Feline Hepatology



Rob and Roxann Bilger (pictured at far right) are the guardians of several cats. After one of their beloved cats, Lilly (pictured at left), developed chronic enteropathy, Rob and Roxann realized that the current state of knowledge in feline gastroenterology is painfully lacking. As a result, they decided to partner with the GI Lab in creating two endowed chairs.

Dr. Jonathan Lidbury (pictured to right), who has been with the GI Lab for many years and who has developed a program in small animal hepatology, has been named the Rob and Roxann Bilger Chair in Feline Hepatology. Together with other team members, he will further investigate liver diseases in cats. One of the first projects will be focused on investigating the role of hepadnavirus in chronic hepatobiliary disease in cats. Hepadnavirus is a virus very similar to human hepatitis B virus and has only recently been identified in cats by investigators in Australia. The impact of this new virus on feline hepatobiliary disease in the USA is poorly understood to date.



Recently, Dr. Lidbury and his team were joined by a new graduate student from Taiwan, Dr. Min-Chun Chen, who will work on this project. We would like to thank Rob and Roxann for their generous support of such important research. (Joerg Steiner)

Serum Submissions

Assay	Vol. req'd	Price
TLI, PLI, Cobalamin, Folate, Cortisol (dogs only)	2.0 ml fasted	$85.00
TLI, PLI, Cobalamin, Folate	2.0 ml fasted	$76.00
TLI, Cobalamin, Folate	1.0 ml fasted	$55.00
PLI, Cobalamin, Folate	1.0 ml fasted	$55.00
TLI, PLI	1.0 ml fasted	$55.00
Cobalamin, Folate	1.0 ml fasted	$38.00
TLI	1.0 ml fasted	$29.00
PLI Note: Spec cPL or Spec fPL test is only offered as part of a panel or alone as a follow-up	1.0 ml fasted	$29.00
Canine C-reactive Protein	0.5 ml fasted	$31.00
Bile Acids	Pre-feeding: 1.0 ml fasted	$18.00
	2 hrs post-feeding: 1.0 ml	$18.00
Methylmalonic Acid	0.5 ml fasted	$56.00
Gastrin	0.5 ml fasted	$29.00
Triglycerides	0.5 ml fasted	$16.00

Fecal Submissions

Assay	Price
Canine Alpha-1 Proteinase Inhibitor Note: A set of 3 fecal samples must be submitted in pre-weighed tubes for testing. Email *gilab@cvm.tamu.edu* to order fecal α_1PI collection tubes (15 for $25.00).	$54.00
Dysbiosis Index: Canine or Feline	$48.00
Canine Enteropathogen Panel Canine panel includes PCR testing for *Clostridium perfringens* enterotoxin gene, net F toxin gene-*C. perfringens*, *C. difficile*, *Campylobacter jejuni*, canine parvovirus, *Salmonella* spp., and IFA testing for *Giardia* and *Cryptosporidium*	$110.00
Feline Enteropathogen Panel Feline panel includes PCR testing for *Clostridium perfringens* enterotoxin gene, net F toxin gene-*C. perfringens*, *C. difficile*, *Campylobacter jejuni*, feline panleukopenia virus (FPV), *Salmonella* spp., *Tritrichomonas foetus*, and IFA testing for *Giardia* and *Cryptosporidium*	$120.00
Real-time PCR Assays First PCR assay Each additional PCR assay *Tritrichomonas foetus*, *Campylobacter jejuni* and *C. coli*, *Heterobilharzia americana*, canine parvovirus (CPV-2), feline panleukopenia virus (FPV), *Salmonella* spp., net F toxin gene-*C. perfringens*	 $36.00 $12.00
Immunofluorescence Assay (IFA) for *Giardia* and *Cryptosporidium*	$38.00
Bacterial Toxin Assays (ELISA) *Clostridium difficile* Toxin A and B *Clostridium perfringens* enterotoxin	 $34.00 $34.00

Sample submission forms customized with your clinic's information are available on our website at ***https://vetmed.tamu.edu/gilab***. Click the "Clinic Login" button. For any questions or to set up a new account, please contact us at (979) 862-2861 or ***gilab@cvm.tamu.edu***.

Hypoadrenocorticism in Dogs

Hypoadrenocorticism, also known as Addison's disease (AD), is an endocrine disorder of the adrenal glands, which predominantly affects young to middle-aged dogs. While both sexes can be affected, females are overrepresented. Addison's disease results from insufficient production of one or more adrenal hormones. There are two forms of AD, known as typical and atypical. Typical AD refers to hypoadrenocorticism that is associated with a typical clinical presentation and abnormalities in serum or plasma potassium and sodium concentrations, whereas atypical AD is not associated with such changes (i.e., lack of electrolyte abnormalities). The most common cause of hypoadrenocorticism is immune-mediated destruction of the adrenal cortex.

The most common clinical signs of AD are somewhat dependent on which form is affecting that particular patient. Dogs with typical AD are commonly presented to the veterinarian for one or more of the following clinical signs: lethargy, polyuria/polydipsia, regurgitation, diarrhea or soft stools (predominantly small intestinal in presentation), vomiting, weakness, weight loss, and potentially collapse and/or hypovolemic shock.

For atypical AD, many of the clinical signs mimic those of dogs affected by chronic enteropathies. A recent study looking at dogs diagnosed with AD compared to a large population of dogs with chronic enteropathies found no difference in the type of presenting clinical signs between Addisonian dogs and those with chronic enteropathies.[1] These included overt signs of upper GI bleeding and, in a few dogs, even evidence of large bowel disease (e.g., hematochezia, tenesmus). As AD does not typically cause large bowel diarrhea, it is unclear if clinical signs in those few dogs were solely from hypoadrenocorticism or another concurrent disease process. Importantly, many of these dogs lacked the "classical" electrolyte abnormalities that develop due to mineralocorticoid deficiency (i.e., hyperkalemia, hyponatremia). This emphasizes the point that **veterinarians should be suspicious of AD in dogs with chronic GI signs, regardless of the presence or absence of electrolyte abnormalities.**

Clinicopathologic abnormalities on a complete blood count (CBC), biochemistry panel, and urinalysis that should raise suspicion for AD include the following*:	
Anemia secondary to GI bleeding[1,2]	Hypoglycemia
Lack of a stress leukogram or "inverse" stress leukogram (i.e., more than 2,500 lymphocytes or more than 500 eosinophils)	Inappropriate urine concentrating ability as evidenced by urine specific gravity (USG) < 1.025
Hypercalcemia	Hyponatremia
Hypokalemia	Azotemia
Hypoalbuminemia and/or hypocholesterolemia (consistent with a protein losing enteropathy)[2]	
*For cases with atypical AD, lack of these abnormalities is common and should not decrease the index of suspicion for this disease.	

Interestingly, the study referenced above also found no significant differences in routine laboratory abnormalities between dogs with AD and those with other causes of chronic GI signs.[1] This again emphasizes the point that **chronic GI signs, along with an appropriate patient signalment, warrants screening for AD**.

Failure to promptly diagnose and treat hypoadrenocorticism might be life-threatening. In stable cases, **AD can be reliably and cost-effectively ruled out using a baseline serum cortisol concentration.** When the baseline cortisol is ≥ 2.0 µg/dL, AD can be ruled out. For dogs in which the resting cortisol is < 2.0 µg/dL, an ACTH stimulation test must be performed for definitive diagnosis. It is recommended that synthetic ACTH (e.g., Cortrosyn, Cosyntropin Injection, and/or Synacthen) is used rather than compounded formulations of ACTH because of the former's more consistent timing of peak action. A recent study concluded that a dose of 1 µg/kg of synthetic ACTH produces an equivalent response to that observed with a dose of 5 µg/kg traditionally used for the diagnosis of hypoadrenocorticism in dogs.[3]

Because of the importance of screening for AD in dogs with chronic GI signs, **we now offer an extended GI panel that adds a baseline cortisol to our standard panel (cPLI, cTLI, cobalamin, and folate) for only $9 more. As we save leftover serum for several weeks, it is also often possible to add a baseline cortisol to testing at a later date – just call to ask about this.** Measurement of serum cortisol in our lab requires a minimum sample volume of 200 µL. It is recommended that serum be separated and shipped in a red top tube with a cold pack. Of note, since hypoadrenocorticism is exceedingly rare in cats, we currently do not recommend measuring a baseline cortisol concentration in cats with signs of GI disease. (Emily Gould)

Addison's disease has been recognized to be more common in several breeds, including, but not limited to, the following:	
Bearded collie	Nova Scotia duck tolling retriever
Cairn terrier	Pomeranian
Cocker spaniel	Portuguese water dog
Great Pyrenees	Standard poodle
Leonberger	West Highland white terrier

References

1. Hauck C, Schmitz SS, Burgener IA, et al. Prevalence and characterization of hypoadrenocorticism in dogs with signs of chronic gastrointestinal disease: A multicenter study. *Journal of Veterinary Internal Medicine* 2020;34:1399-1405.
2. Wakayama JA, Furrow E, Merkel LK, et al. A retrospective study of dogs with atypical hypoadrenocorticism: a diagnostic cut-off or continuum? *The Journal of Small Animal Practice* 2017;58:365-371.
3. Botsford A, Behrend EN, Kemppainen RJ, et al. Low-dose ACTH stimulation testing in dogs suspected of hypoadrenocorticism. *Journal of Veterinary Internal Medicine* 2018;32:1886-1890.

Feline Dysbiosis Index

We are pleased to announce that we can now also offer a feline dysbiosis index (DI) to assess the fecal microbiota of cats.[1] While the principle behind this assay is similar to the canine DI,[2] the feline assay evaluates a few other bacterial taxa that are more specific to identify fecal dysbiosis in cats (*Table 1*). The feline DI has been established based on fecal samples obtained from 80 healthy pet cats and 68 cats with chronic enteropathy (CE).[1] When the cut-off value of the DI was set at 0, it provided 77% sensitivity and 96% specificity to differentiate the microbiota of cats with CE from those of healthy cats.

Table 1. Reference Intervals

	Abundance	Change in dysbiosis
Faecalibacterium	3.8 - 8.4	Decreased
Turicibacter	4.4 - 9.0	Decreased
Clostridium hiranonis	4.5 - 7.1	Decreased
Bifidobacterium	3.2 - 8.7	Decreased
Bacteroides	4.0 - 7.5	Decreased
Streptococcus	1.6 - 5.2	Increased
E. coli	1.4 - 7.0	Increased

Data expressed log DNA/gram of feces

Interpretation

The DI should be interpreted together with the abundance of the individual bacterial taxa. A DI above 1 indicates a major shift and dysbiosis with high specificity, while a DI between 0 and 1 indicates a moderate shift in the fecal microbiome (*Figure 1*). Some cats with CE have a DI < 0, but with some bacterial taxa outside the reference intervals. This suggests a minor form of dysbiosis.

Before assessing the microbiome, cats should be off treatment with omeprazole and/or antibiotics. Omeprazole leads to a transient increase in the DI, which normalizes within two weeks after discontinuation of therapy.[3] Antibiotics also induce fecal dysbiosis. The microbiota typically normalizes within two to four weeks after discontinuation of administration in most cats, but some may have a persistent dysbiosis.[4]

The DI can be especially useful to evaluate healthy cats as potential donor for fecal microbiota transplantation (FMT)[5], as a small subset of clinically healthy cats may have a shift in the microbiota (*Figure 1*). Also, cats with non-specific clinical signs (i.e., lack of diarrhea and/or vomiting) may have an increased DI suggesting the presence of chronic enteropathy (*Figure 2*). More information about microbiota dysbiosis can be found at https://tx.ag/DysbiosisGI. (Jan Suchodolski)

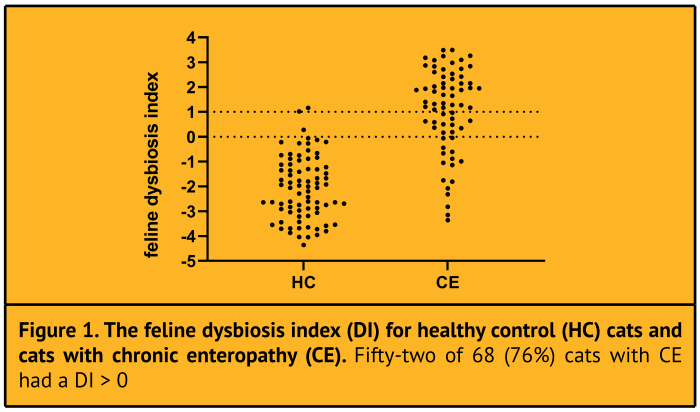


Figure 1. The feline dysbiosis index (DI) for healthy control (HC) cats and cats with chronic enteropathy (CE). Fifty-two of 68 (76%) cats with CE had a DI > 0

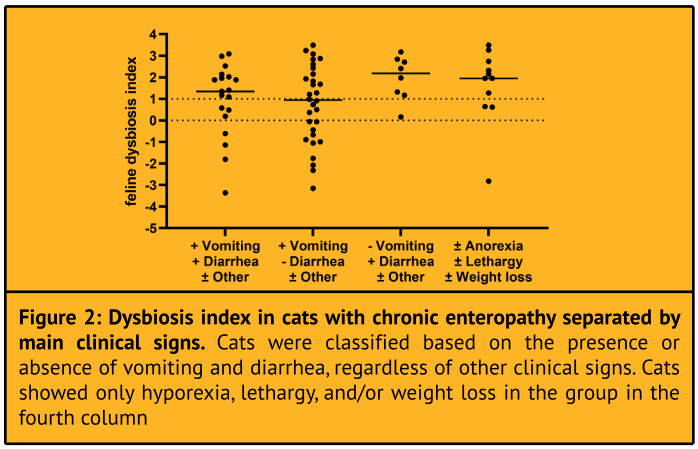


Figure 2: Dysbiosis index in cats with chronic enteropathy separated by main clinical signs. Cats were classified based on the presence or absence of vomiting and diarrhea, regardless of other clinical signs. Cats showed only hyporexia, lethargy, and/or weight loss in the group in the fourth column

References

1. Sung CH, Marsilio S, Chow B, et al. Dysbiosis index to evaluate the fecal microbiota in healthy cats and cats with chronic enteropathies. *J Feline Med Surg* 2022:1098612X221077876.
2. AlShawaqfeh MK, Wajid B, Minamoto Y, et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiol Ecol* 2017;93:doi: 10.1093/femsec/fix1136.
3. Schmid SM, Suchodolski JS, Price JM, et al. Omeprazole minimally alters the fecal microbial community in six cats: a pilot study. *Front Vet Sci* 2018;5:79.
4. Torres-Henderson C, Summers S, Suchodolski JS, et al. Effect of Enterococcus faecium strain SF68 on gastrointestinal signs and fecal microbiome in cats administered amoxicillin-clavulanate. *Top Companion Anim Med* 2017;32:104-108.
5. Chaitman J, Gaschen F. Fecal microbiota transplantation in dogs. *Vet Clin North Am Small Anim Pract* 2021;51:219-233.

SAVE THE DATE:
Vet Ku - Texas A&M University 2023 Internal Medicine Conference
Focus: Nephrology and Urology

We are very excited to announce the 2023 Internal Medicine Conference in partnership with the Faculty of Veterinary Medicine at Kasetsart University in Bangkok, Thailand. The conference will be held at a family-friendly, five-star resort in Pattaya, Thailand, in **October 2023** (dates TBA). The focus will be nephrology and urology. A panel of internationally renowned experts will deliver 25 hours of top-quality continuing education. More details to follow…

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Current studies / Contact information	Brief project description
Comparison of parenteral and oral cobalamin supplementation in cats Dr. Chee-Hoon Chang chchang@cvm.tamu.edu	This project aims to compare the efficacy of parenterally and orally administered cobalamin supplementation in **cats**. Cats with cobalamin deficiency for any reason can be enrolled. However, patients cannot have any significant comorbidities, such as chronic kidney disease. There is no cost to the owner for participation other than for office visits.
Treatment trial for canine chronic pancreatitis Dr. Sue Yee Lim slim@cvm.tamu.edu	The aim of this clinical trial is to assess the efficacy of cyclosporine or prednisolone for treating chronic pancreatitis in **dogs**. Patients will receive prednisolone or cyclosporine for the three weeks of the study at no charge as well as GI panels.
Dietary management for chronic pancreatitis Dr. Floris Droees fdroees@cvm.tamu.edu	The aim of this study is to evaluate the efficacy of an ultra-low fat diet for **dogs** with chronic pancreatitis. The study will provide the diet free of charge for the duration of the study as well as monitoring of cPLI concentrations.
Treatment trials for feline chronic pancreatitis Dr. Yu-An (Andy) Wu yuanwu@cvm.tamu.edu	We are enrolling cats into two studies of chronic pancreatitis: (1) Symptomatic treatment only -- The aim of this study is to collect data from **cats** with chronic pancreatitis that are NOT being treated with an immunosuppressive drug (e.g., prednisolone or cyclosporine). (2) Effacacy of cyclosporine -- The aim of this case series is to assess the efficacy of cyclosporine for treating chronic pancreatitis in **cats**. Costs of the cyclosporine will be reimbursed. Both studies include a total of 3 visits (initial appointment, 10th day, and 21st day). Shipping and GI panels are covered.
Evaluation of markers of pancreatic disease in cats before and after switching to a special diet for kidney disease or diabetes mellitus (The CATPAD study) Dr. Yu-An (Andy) Wu yuanwu@cvm.tamu.edu	The CATPAD study is a project that looks at cats' pancreatic health and the possible association with diet. We are currently enrolling **cats** that are about to be switched to a commercially available therapeutic diet intended for cats with kidney disease or diabetes mellitus. More information is available at: https://vetmed.tamu.edu/gilab/research/catpad-study/.
Evaluation of anti-inflammatory and cytotoxic properties of acid suppressants on canine resectable mast cell tumors (MCTs) Dr. Emily Gould egould@cvm.tamu.edu	Study aims are to evaluate blood and tissue cytokines, MCT viability, and quantifiable histamine (and/or histamine metabolites) before and after acid suppressant or placebo therapy in **dogs** with surgically resectable MCTs. Study includes a total of 3 visits (initial appointment, surgical resection of tumor, and one post-operative recheck).