The GI Lab

VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY

Promoting Gastrointestinal Health in Companion Animals

Newsletter - Spring 2016 Editors: JM Steiner, JA Lidbury & JS Suchodolski

What's inside:

The Gastrointestinal Laboratory at Texas A&M University

News from the GI Lab

Another year has passed and it is time to give you a brief update on our operations. In order to strengthen our leadership team we have divided responsibilities. Jan Suchodolski will now serve as the Associate Director for Research and Chief of Microbiome Science. Jonathan Lidbury will serve as Associate Director for Clinical Services. Of course you can continue to call upon all of us if you need anything.

We have also strengthened our team of clinical consultants. Dr. David Williams (University of Illinois) and Dr. Craig Ruaux (Oregon State University) will continue to serve as external consultants for clinical consults. Locally, consulting responsibilities are shared between Dr. Jonathan Lidbury, Dr. Sina Marsilio, Dr. Yuri Lawrence, and myself. All of us are board-certified internists with a long-standing interest in GI disease, so we hopefully will be able to continue to serve your needs for clinical consults on difficult cases.
Also, in April of this year we were able to move into our new genetics lab – we believe a state-of-the-art facility that will enhance our abilities to do microbiome research. We continue to remodel our remaining space to optimally serve our needs. As always, thank you for your continued patronage – without your continued support we would not be able to do the work we do – thank you!!! (Jörg M. Steiner)

Jan Suchodolski, MedVet, DrMedVet, PhD, DACVM, AGAF Associate Professor & Associate Director for Research, Chief Microbiome Science

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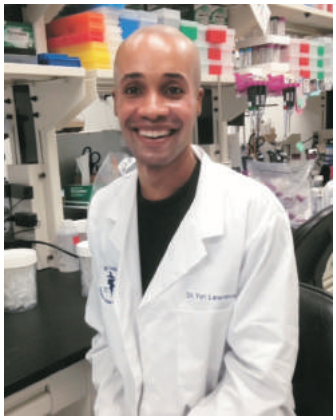

Yuri Lawrence, DVM, MA, MS, DACVIM

Confused about lipase assays?

Key Facts

- **During pancreatitis, pancreatic lipase is released into the bloodstream and can be used as a diagnostic marker for the disease**
- **Lipases are also released from other organs than the pancreas, adding to the total serum lipase activity**
- **Spec cPL® and Spec fPL® specifically measure lipases of pancreatic origin, making them very sensitive and specific for a diagnosis of pancreatitis**
- **DGGR- and triolein-based lipase assays measure total serum lipase activity and thus are not specific for the measurement of pancreatic lipase**

Acute and chronic pancreatitis are now recognized to be common in both dogs and cats, but their diagnosis remains to be challenging. Various diagnostic tests have been used to confirm a diagnosis of acute and chronic pancreatitis, including the measurement of serum lipase activity, measurement of serum pancreatic lipase immunoreactivity (PLI), abdominal ultrasound, and histopathology.

Assays for the measurement of serum lipase activity were commonly used in the past because they were minimally invasive, inexpensive, and did not require special equipment or expertise. Although the exocrine pancreas is a major source of serum total lipase activity, it is not the only organ or type of cell that produces, stores, and releases a lipase. Lipase may be released by the stomach (gastric lipase), the liver (hepatic lipase), endothelium (endothelial lipase), and by many other organs and cells. All of these lipases contribute to the total lipase activity measured in serum. Thus, assays for the measurement of serum total lipase activity lack specificity for the pancreas. In addition, they are not specifically sensitive for the diagnosis of pancreatitis. Depending on the cutoff value used, serum total lipase activity has been reported to have a sensitivity as low as 13.6% for macroscopic pancreatitis, thus missing up to 86.4% of patients with pancreatitis. The sensitivity and specificity naturally changes with different cut-off values. This explains why some studies found higher sensitivities (up to 71%) but at the expense of specificity with an increasing number of false-positive results (specificity 43%, i.e., 57% of positive results are false-positive). Furthermore, renal, gastrointestinal, hepatic, and neoplastic disease, as well as steroid administration have all been shown to cause increases in serum total lipase activity.

Serum pancreatic-lipase immunoreactivity (PLI) as measured by the Spec cPL® or Spec fPL® assays specifically measure lipase that originates from the acinar cells of the canine or feline pancreas, respectively. These assays use antibodies directed against pancreatic lipase and to date no other lipase has been shown to cross-react with these antibodies. Therefore, Spec cPL and fPL are considered to be the most specific diagnostic tests for the exocrine pancreas. Also, measurement of Spec PL® is highly sensitive for a diagnosis of pancreatitis in both dogs and cats. In dogs with clinically significant signs of pancreatitis, Spec cPL has been shown to identify the disease with a sensitivity of 82 to 94%.[1] In dogs with less severe pancreatitis, Spec cPL still showed the highest accuracy among any diagnostic test with a sensitivity of 64%. In cats, Spec fPL correctly identified patients with pancreatitis with a sensitivity between 54% (subclinical to mild disease) to 100% (moderate to severe disease).[16] Also, specificities for Spec cPL and fPL have been reported to be between 79 and 100%.[1,10,16]

Recently, two substrates for the measurement of total serum lipase activity (triolein and 1,2-o-dilauryl-rac-glycero-3-glutaric acid (-6'-methylresorufin, DGGR)) have been marketed for the measurement of total serum lipase activity. Both substrates are used in catalytic assays, where serum lipases cleave the substrate and products of this reaction are measured via colorimetry. The v-LIP-P slide detects serum lipases using triolein as the substrate and a negatively charged detergent as an auxiliary agent, while the DGGR-lipase assays use DGGR as a substrate. Both substrates have been claimed to have a comparable diagnostic utility to that of serum Spec PL.

In 2005, Graca et al. validated a DGGR lipase assay for use in dogs. Their study showed, that using a cut-off value of 120 U/L, this test had a high sensitivity of 93%, but poor specificity of 53%. When the cut-off value was increased to 180 U/L, the specificity slightly improved reaching 66%, whereas sensitivity decreased to 73%. The authors of that study discussed the possibility of cross-reactivity of non-pancreatic lipases with the substrate. Indeed, a recent in-house study indicates that DGGR is hydrolyzed by other enzymes (likely non-pancreatic lipases; unpublished data). Serum lipase activity in leftover serum samples of 48 dogs with exocrine pancreatic insufficiency (TLI < 1µg/L) and 66 healthy control dogs was measured using a DGGR-based assay (Diazyme Laboratories, Poway, CA and Stanbio Laboratory Boerne, TX). Our data showed that serum lipase activity was within the reference interval in 33 of 48 dogs with EPI using a DGGR-based assay (Figure 1).

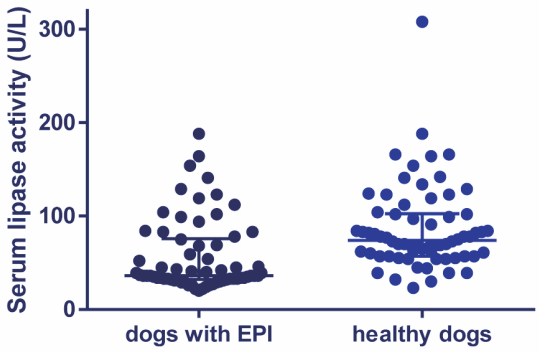

Figure 1. Serum lipase activities in dogs with EPI and healthy control dogs as measured by an assay utilizing DGGR as a substrate. The center lines show the medians for each group and the whiskers display the interquartile ranges. Serum lipase activity measured by a DGGR-based assay was readily detectable in many dogs with EPI. Thus, DGGR-based assays for the measurement of serum lipase activity are not specific for pancreatic lipase.

Confused about lipase assays? - continued

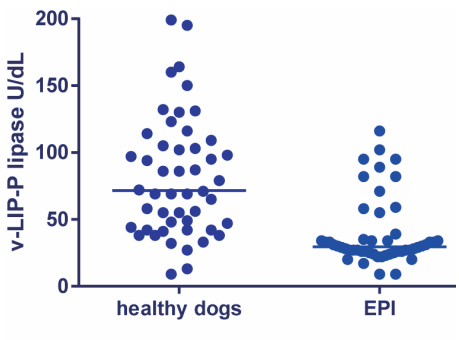

Figure 2: Lipase activity as measured by the v-LIP-P slide in 50 healthy dogs and 50 dogs with EPI. Many of the dogs with EPI had significant serum lipase activities. The lines indicate the medians for both groups.

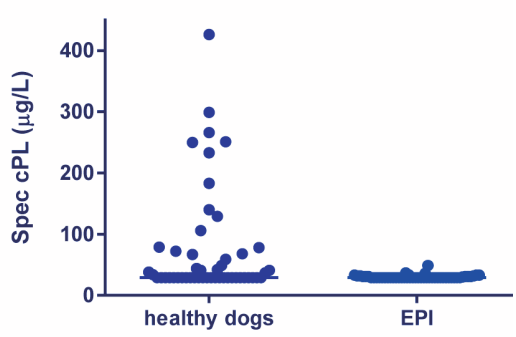

Figure 3: Pancreatic lipase immunoreactivity as measured by Spec cPL in 50 healthy dogs and 50 dogs with EPI. There is much better separation between healthy dogs and dogs with EPI than for the v-LIP-P slide. The lines indicate the medians for both groups.

Similarly, we compared serum lipase activity measurements in leftover serum samples from 50 dogs with EPI (TLI < 2.5 µg/L) and 50 healthy control dogs using the v-LIP-P slide (FujiFilm Corporation, Tokyo, Japan) and the Spec cPL (IDEXX Laboratories, Inc., Westbrook, Maine, USA). While the serum Spec cPL was in the lower 20% of the reference interval in 49 of 50 (98%) dogs with EPI, serum lipase activity measured with the v-LIP-P slide was in the lower 20% of the reference interval in only 29 of 58 EPI dogs (58%), indicating the detection of lipases other than that of pancreatic origin (Figures 2 and 3). These results clearly show that, in contrast to Spec cPL, triolein and DGGR are not specific for the measurement of pancreatic lipase.

Several studies have been published that directly compared the aforementioned assays with the Spec cPL or fPL. All of these studies found good to moderate correlation between the assays. However, while it is intuitive to assume that serum lipase activity increases as the fraction of pancreatic lipase in patients with pancreatitis and that all aforementioned assays will detect that increase in activation, a correlation between tests is not equivalent to a test having the same sensitivity and specificity and thus discriminatory power between disease and controls. A recent study found a moderate agreement (Cohens value 0.803) between a DGGR lipase assay and Spec cPL when a 2-fold DGGR lipase "gray zone" was applied with cut offs of 216 U/L for DGGR and 400 µg/L for Spec cPL. Sensitivity and specificity could not be calculated since pancreatic histopathology was not available. Another study compared serum lipase activity as measured with the v-LIP-P slide with the measurement of pancreatic lipase using the Spec cPL and showed good correlation (r=0.91). However, when looking at the data in detail, the assay showed good correlation only for lower lipase values, but showed an increasing variation with increasing values, starting at about 400 µg/L Spec cPL (cutoff for pancreatitis).[11] (see Ishioka, K., Hayakawa, N., Nakamura, K. & Terashima, K. Patient-side assay of lipase activity correlating with pancreatic lipase immunoreactivity in the dog. J. Vet. Med. Sci. 73, 1481–1483; 2011). This indicates non-constant variances (heteroscedasticity) and thus a simple correlation does not reflect the assays' true diagnostic value.

A recent study comparing the DGGR lipase assay (cutoff > 26 U/L) with Spec fPL (> 3.4 µg/L) in cats with acute or chronic pancreatitis also showed moderate agreement between the two assays (Cohen´s value 0.601). Based on histopathology, sensitivity and specificity were 48% and 63% for the DGGR lipase assay and 57% and 63% for Spec fPL, respectively. However, a previous study showed higher sensitivities and specificities for Spec fPL in cats with different stages of pancreatitis. In cats, with histopathologically confirmed mild pancreatitis, sensitivity of Spec fPL was 54%, while this percentage increased to 100% in cats with moderate to severe pancreatitis. Specificity of Spec fPL has been as high as 100% when tested against a healthy control group.

In addition to significant problems with sensitivity and specificity, assays for total serum lipase activity have been shown to be greatly influenced by hemolysis, lipemia, and icterus, influence factors that are frequently observed in dogs with pancreatitis and/or other gastrointestinal or hepatic diseases. In an in-vitro study, we recently evaluated the effects of these factors on the performance of lipase activity assays using the substrate triolein, and on the performance of the Spec cPL ELISA assay. Serum samples that were spiked with canine hemoglobin, Intralipid®, or synthetic ditaurobilirubin, mimicking hemolysis, lipemia, or icterus, respectively (unpublished data). (continued next page)

Effect of influence factors on results of lipase assays

The triolein-based lipase assay v-LIP-P slide is highly influenced by hemolysis, lipemia, and icterus

In contrast, hemolysis, lipemia, and icterus have no effect on results of the Spec cPL® assay

Confused about lipase assays? - continued

Evaluation of the v-LIP-P slide showed that this assay significantly underestimates serum lipase activity in hemolyzed and icteric samples, thus limiting sensitivity of the assay in these samples. This is likely to translate into a high false-negative rate in patients with pancreatitis, i.e., missing a diagnosis of pancreatitis in some patients. In lipemic samples the use of triolein dramatically increased the reported lipase activity, potentially leading to a dramatically increased rate of false-positive diagnosis of pancreatitis (poor specificity). In fact, even mild lipemia (serum triglyceride concentrations of approximately 300 mg/dL), which would not be expected to be identified by gross examination of the serum sample, showed dramatic effects with 9 of 10 samples generating results above the working range of the assay (Figure 4). In contrast, hemolysis, lipemia, and icterus at the same levels showed no effect on the performance of the Spec cPL assay. These results show, that the Spec cPL and fPL are superior for the diagnosis of pancreatitis in dogs and cats to any lipase activity assay, regardless of the substrate that is being used. (Sina Marsilio and Jörg M. Steiner)

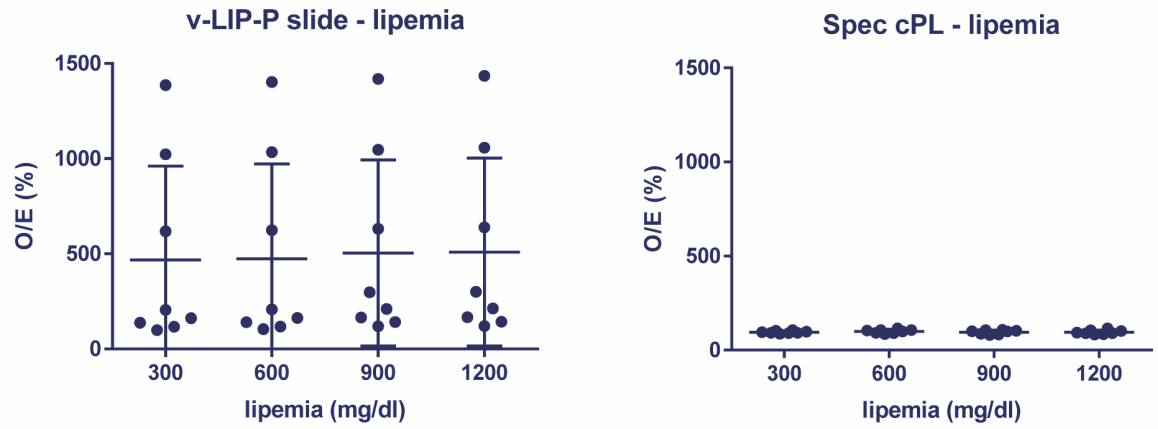

Figure 4: Observed to expected ratios for serum samples spiked with Intralipid.

Serum samples spanning the working ranges for the v-LIP-P slide and the Spec cPL assay were spiked with 300, 600, 900, or 1,200 mg/dL of Intralipid. Observed to expected ratios increased dramatically when samples were analyzed with the v-LIP-P slide (scatterplot on the left) showing that the assay drastically overestimates serum lipase activities in even mildly lipemic samples. In contrast, Intralipid had no effect on the Spec cPL assay (scatterplot on the right).

Conclusion

Spec cPL® and Spec fPL® remain superior to any activity assay for the measurement of serum lipase activity for the diagnosis of pancreatitis in both dogs and cats.

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A Paradigm Shift for Cobalamin Supplementation

Cobalamin (vitamin B12) deficiency occurs commonly in both dogs and cats with chronic gastrointestinal disease. Traditionally, cobalamin has been supplemented by parenteral administration, but recent data suggests that oral cobalamin supplementation may be as efficacious as parenteral supplementation. Given the ease of oral administration this mode of supplementation may be preferable to the parenteral route.

Cobalamin Deficiency

The most common causes of cobalamin deficiency in dogs and cats are chronic and severe distal or diffuse small intestinal disease and exocrine pancreatic insufficiency (EPI). In addition, short-bowel syndrome, an exclusively vegetarian or vegan diet, or hereditary cobalamin deficiency are less common causes of cobalamin deficiency. Most dogs and cats with cobalamin deficiency only show clinical signs of gastrointestinal disease, which could either be a cause or the effect of cobalamin deficiency. Other clinical signs include weight loss, central neuropathies, peripheral neuropathies, or immunodeficiencies.

Reaching a definitive diagnosis of cobalamin deficiency can be challenging. Clinical signs are ultimately caused by cobalamin deficiency on a cellular level. However, the cellular cobalamin status is difficult to assess. Serum cobalamin concentration has been traditionally measured to help assess cobalamin status, but some patients with cobalamin deficiency on a cellular level do not have severely decreased serum cobalamin concentrations. Thus, in order to avoid missing cobalamin-deficient patients, cobalamin supplementation should be considered even when serum cobalamin concentration is in the low end of the reference interval. Several assays for the measurement of serum concentrations of cobalamin in humans are available. In order to be used in dogs and cats, these assays must be validated for use in dogs and cats. The GI Lab at Texas A&M University has analytically validated an automated chemiluminescence assay designed for the measurement of cobalamin concentrations in humans for use in dogs and cats. Reference intervals for serum cobalamin concentration in dogs and cats have been established and are updated periodically. Reference intervals are not transferrable between labs and each lab should establish their own reference interval.

Serum or urine methylmalonic acid (MMA) concentration can also be used as an indicator of cobalamin status. Cobalamin deficiency leads to accumulation of MMA and thus concentrations of MMA are often dramatically increased in the serum or urine of patients with cobalamin deficiency. In fact, measurement of serum MMA concentration may be a better diagnostic test for cobalamin deficiency than serum cobalamin concentration because it allows assessment of the metabolic state of cells in response to a lack of cobalamin. However, measurement of MMA concentration in serum or urine is technically involved and expensive. Thus, serum/urine MMA concentrations are not routinely assessed in patients being evaluated for cobalamin deficiency, but can be measured at the GI Lab when needed. In summary, the only routinely available diagnostic tool to assess cobalamin status in dogs and cats is measurement of serum cobalamin concentration. This should be evaluated in every dog and cat with chronic signs of gastrointestinal disease or with clinical signs compatible with cobalamin deficiency that cannot be attributed to other conditions (i.e., unexplained immunodeficiencies, anemias, neuropathies).

Cobalamin Supplementation

Patients with severe cobalamin deficiency often do not respond to therapy of their underlying gastrointestinal disorder unless or until cobalamin is supplemented. As mentioned, cobalamin supplementation should be considered in patients with low-normal serum cobalamin concentrations, as measurement of serum cobalamin concentration may not be optimally sensitive for the diagnosis of cobalamin deficiency and there is no indication that over-supplementation of cobalamin leads to complications. The most common form of cobalamin used for supplementation is cyanocobalamin, but hydroxocobalamin or methylcobalamin can also be used in patients that don't respond to cyanocobalamin supplementation (most of these patients will also fail to respond to other forms of cobalamin) or those that appear to have side effects to supplementation with cyanocobalamin (side effects from cyanocobalamin administration have never been definitively demonstrated in either dogs or cats). Traditionally, the standard route of cobalamin application is by subcutaneous injection. This is because cobalamin deficiency has been shown to lead to cobalamin malabsorption in the ileum. However, there are recent data that show that oral supplementation may be as efficacious as parenteral supplementation. The dosing schedule for parenteral supplementation consists of 250 µg cobalamin in cats and 250-1500 µg in dogs, administered subcutaneously once a week for 6 weeks, followed by one more dose a month later, and re-evaluation a month after that. As for parenteral administration, dosing for oral supplementation is empiric with 250 µg of cyanocobalamin being administered orally once a day in cats or in dogs up to 10 kg BW, 500 µg in dogs weighing over 10 kg but less than 20 kg, and 1000 µg in dogs weighing more than 20 kg. Daily supplementation is required and after a treatment period of approximately 3 months one should discontinue supplementation for a week and recheck serum cobalamin concentration.

In one large retrospective study of 51 client-owned dogs with low-normal or subnormal serum cobalamin concentrations patients were supplemented with oral cyanocobalamin (250-1000 µg cobalamin orally once a day) and serum cobalamin concentrations increased in all of the dogs. Interestingly, not all patients had the same underlying cause of cobalamin deficiency, suggesting that the cause of cobalamin deficiency may not play a role in determining the success of oral supplementation. Similarly, a more recent small retrospective study in 16 cats with chronic enteropathy or intestinal lymphoma and low or low-normal serum cobalamin concentrations showed dramatic increases in serum cobalamin concentrations in all 16 cats. While prospective studies are needed and ongoing, these initial data are very promising and oral supplementation could be applied routinely unless there is evidence that a particular patient does not respond to this route of supplementation. (Jörg M. Steiner)

Injectable cyanocobalamin. There are many generic formulations of injectable cobalamin. Formulations most commonly used are composed of cyanocobalamin at 1000 µg/ml.

Cyanocobalamin. This picture shows chewable cobalamin tablets for use in dogs and cats.
Courtesy of Nutramax Laboratories Veterinary Sciences, Inc.

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Dysbiosis Index – a New Tool to Assess the Gastrointestinal Microbiota

The intestinal microbiota consists of viruses, bacteria, fungi, and protozoa. Molecular methods are now the standard for the identification of intestinal bacteria. An estimated 100 trillion microbial cells are present in the GI tract, which is approximately 10 times more than the number of host cells. This complex ecosystem of gut bacteria has a tremendous influence on host health. A balanced microbiota regulates the immune system, helps in the defense against enteropathogens, and provides nutritional benefits. Interactions between intestinal bacteria and the host immune system are mediated through direct contact between microbes and the immune system (e.g., dendritic cells, Toll-like receptors), and through microbiota derived metabolites. Anaerobic bacteria, such as *Ruminococcus*, *Faecalibacterium*, *Lachnospiraceae*, *Collinsella*, and *Clostridiales* produce metabolites that have direct beneficial effects on the host. For example, nutrient sources such as complex carbohydrates (e.g., starch, cellulose, pectin) are fermented by bacteria, resulting in the production of short chain fatty acids (SCFA). These act as energy sources for the host, regulate intestinal motility, and are important growth factors for epithelial cells. SCFA also have direct anti-inflammatory properties through expansion of immunoregulatory lymphocytes. Other bacterially derived metabolites such as indole, a byproduct of tryptophan degradation, or secondary bile acids, are also immuno-modulatory, thereby maintaining immune homeostasis and strengthening intestinal barrier function. These beneficial effects of the gut microbiota reach beyond the GI tract. Recent research has shown that alterations in gut microbes play a role in the pathogenesis of diabetes mellitus and obesity.

Dysbiosis

Intestinal dysbiosis, defined as an alteration of the intestinal microbiota composition and/or richness, is associated with acute and chronic GI disorders. A dysbiotic microbiome may be deleterious due to the production of bacterial toxins or due to reductions in anti-inflammatory metabolites derived from bacteria. Dysbiosis may also serve as a risk factor for the development of chronic GI disease in susceptible individuals. For example, antibiotic-induced dysbiosis in early childhood is one of the most important risk factors for the development of allergies, obesity, and IBD in adult humans. These initial data in humans combined with better understanding of the immunomodulatory properties of the gut microbiota emphasizes that proper diagnosis and correction of dysbiosis are important.

Changes in the microbiota result in functional and immunological consequences for the host. For example, mucosa-adherent bacteria in the small intestine are an important stimulator of mucosal immunity, and changes in microbial composition may have significant effects on the host immune response. Dysbiosis may also lead to destruction of brush border enzymes, damage of carrier proteins, and competition for nutrients (e.g., vitamin B12). Also, bacterial enterotoxins stimulate mucosal fluid secretions, resulting in diarrhea. Dysfunction of the mucosal barrier can lead to altered intestinal permeability and clinically significant bacterial translocation. This depletion of commensal groups (Figure 1) in the large intestine and their respective immunoregulatory metabolites (e.g., SCFA, indoles, and secondary bile acids) may impair the ability of the host to down-regulate the aberrant intestinal immune response, making dysbiosis an integral part of the pathogenesis of chronic GI disease.

Diagnosis of dysbiosis

Because of the importance of the physiologic microbiota in maintaining immune homeostasis, it is important to diagnose dysbiosis. Better characterization of dysbiosis may guide treatment decisions as to the need of antimicrobials vs. dietary and/or probiotic therapy and/or immunosuppression. Fecal bacterial culture has no utility for characterization of the many anaerobes in the GI tract. It is currently estimated that less than 20% of intestinal bacteria are cultivable with standard laboratory techniques. Thus, currently the best method to fully characterize the microbiota is by high-throughput sequencing platforms. But due to cost and long turnaround times, this approach is currently only available for research studies. In several studies we have identified the major bacterial groups that are consistently altered in dogs with chronic enteropathies (CE). We have developed PCR based assays that are able to measure the abundances of these bacterial groups (Figure 1). A mathematical algorithm is used to report these changes as a single numerical value; the so called dysbiosis index (DI). A negative DI indicates normobiosis, whereas a positive DI indicates dysbiosis. The DI was specifically trained to diagnose the dysbiosis associated with CE, and an increased DI in dogs with CE provides additional information during the diagnostic work-up of these dogs. The therapeutic approach for these dogs should be in line with the currently recommended series of therapeutic trials (i.e., first a dietary trial, then an antibiotic trial, and finally an anti-inflammatory trial). The DI can be used to monitor the microbiota's response to therapy. Another potential use of the DI is diagnosing primary dysbiosis. As a consequence, it would be reasonable to treat dogs with chronic intermittent diarrhea, which are not systemically ill, and have an increased DI, with therapies aimed at modulating their intestinal microbiota (e.g., probiotics and/or prebiotics). Other uses for the DI are being evaluated in clinical studies; for example, ongoing studies are using it to screen the feces of healthy dogs in order to select donors for fecal transplantations.

The dysbiosis index is now offered by the GI Lab at Texas A&M University. Approximately 0.5 gram of feces are needed, shipped overnight either cooled or frozen. Shipment without ice packs must be avoided. Results will be reported within 2-3 days. (Jan Suchodolski)

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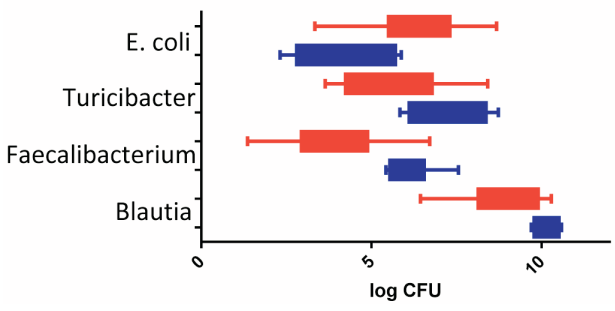

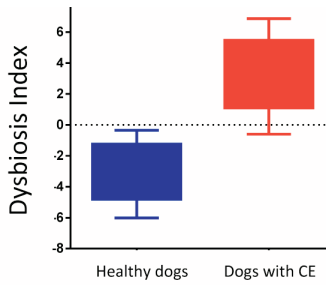

Figure 1. Changes in the major bacterial genera in dogs with CE.

Left: Some bacterial groups are decreased in dogs with CE (red) compared to healthy control dogs (blue), while the abundance of *E. coli* is typically increased. **Right:** The dysbiosis index (DI) is a ratio that combines the changes in bacterial genera (shown on the left) into one single numerical value. A positive dysbiosis index is associated with CE, while a negative DI indicates a normal microbiota.

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Our newly remodeled state-of-the art genetics laboratory