The GI Lab at Texas A&M | Veterinary Medicine & Biomedical Sciences, Texas A&M University

Promoting Gastrointestinal Health in Companion Animals

Newsletter - 2014

Editors: JM Steiner & JS Suchodolski

What's inside:

The Gastrointestinal Laboratory at Texas A&M University

News from the GI Lab

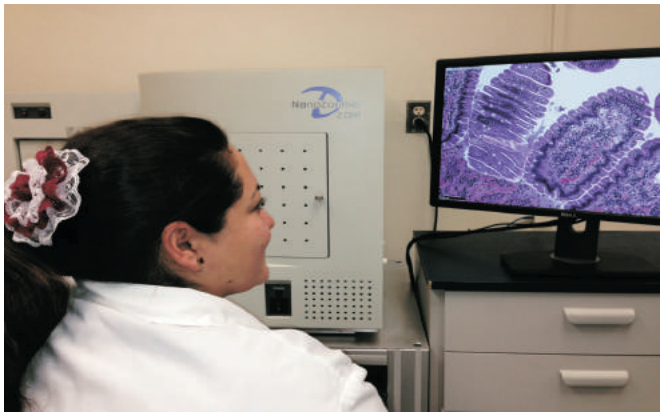
Another year has come and gone and once again it is time to give you a brief update on what is happening at the GI Lab. While I can't report on any new diagnostic assays that we can immediately offer for your daily use, a lot has happened here over the last year.

In December, we were able to take possession of our new slide scanner from Hamamatsu. This may sound like small news, but this new slide scanner allows us to rapidly and automatically scan a large number of histopathology slides that can then be read remotely. We have used a much smaller and slower system for the last 2 years to allow us to work with pathologists with a special interest in the gastrointestinal tract across the globe. However, each case took at least 30 to 60 minutes to scan, limiting the use of the old system to small studies. This new system allows us to work on large studies and also ultimately build a specialized histopathology service for the gastrointestinal tract.

Earlier in the fall we were also able to move into our new service lab, a newly remodeled space that provides us with more space overall, but, more importantly, allows us to establish an improved work-flow, which in turn helps us to improve turn-around times. Our next big facilities project is the remodeling of our genetics lab, which will allow us to streamline our research activities in this area while also allowing us to enhance our offerings for genetic testing for enteropathogens, such as *Tritrichomonas foetus, Heterobilharzia americana* (see later in this issue), pathogenic *Campylobacter* spp., and others.

We continue to work on new diagnostic tests that hopefully will prove to help in the diagnosis and monitoring of gastrointestinal diseases in dogs and cats. We have spent several years investigating markers of hepatic fibrosis, unfortunately with little success. Even markers moderately successful in assessing hepatic fibrosis in humans have not proven to be useful in dogs with hepatic disease. So thequest continues and we have also expanded the search to markers of hepatic inflammation.

Recently, we were able to identify a new marker for eosinophilic inflammation, 3-bromotyrosine, a stable product of eosinophilic oxidation. We are currently in the process to evaluate the clinical utility of this new marker in dogs and cats with gastrointestinal disease. This marks an important step in building a panel for cell-type specific inflammation in the GI tract: methylhistamine for assessment of mast cell inflammation, calprotectin and S100A12 for neutrophilic inflammation, and 3-bromotyrosine for eosinophilic inflammation. The next step will be a search for markers of lymphocytic and/or plasmacytic inflammation. (Jörg M. Steiner)



New scanner for histology slides. This new system allows us to work on large studies and also ultimately build a specialized histopathology service for the gastrointestinal tract.

Consultant Corner

As you probably know, we offer free consultation services for our client veterinarians (we will not consult with pet owners directly). All consultants are board-certified by the American College of Veterinary Internal Medicine and have a special interest in gastrointestinal disease. However, all of us have daytime jobs as well and so sometimes you may not be able to speak to us directly when you call and we end up playing phone tag. In order to decrease your wait time we have decided to start a column in our newsletter where we answer commonly asked questions in hopes that this will save you a phone call.

These questions and answers will also be posted on our website where you can also find some other useful information (www.vetmed.tamu.edu/Gilab; just click on "FAQ" under the service headline). (Jörg M. Steiner)

Do pancreatic enzyme supplements interfere with the TLI assay?

No, both the canine and the feline TLI assays are species-specific and thus these assays will not immuno-cross-react with the porcine trypsinogen/trypsin contained in the pancreatic enzyme supplement.

Are postprandial serum samples acceptable for measurement of serum concentrations of TLI and/or PLI?

It is best to submit serum samples collected at least 12 hours after the last meal as our reference intervals have been established using samples after withholding food. Also, TLI assays are radioimmunoassays and they are susceptible to assay error when a lipemic sample is being used. However, in most cases the changes due to using a sample from a patient from which food has not been withheld for 12 hours will only cause minimal differences that are not clinically relevant. For example, a serum cTLI concentration of 3.5 µg/L (reference interval 5.7-45.2 µg/L; cut-off value for exocrine pancreatic insufficiency: 2.5 µg/L) in a patient from which food has not been withheld for 12 hours or more may be questionable, but a cTLI of 5.7 µg/L or higher is not compatible with a diagnosis of EPI, regardless of feeding status.

My canine patient has a serum cPLI (as measured by Spec cPL®) between 200 and 400 µg/L, what does this mean?

Serum cPLI concentrations between 200 and 400 µg/L are equivocal for pancreatitis. These values are not normal, but are not high enough to arrive at a diagnosis of pancreatitis either. In this situation we suggest that you try to rule out other potential causes of the dogs clinical signs, but still consider pancreatitis as a possibility. We also advise repeating the cPLI measurement in 2 to 3 weeks.

What is the replacement protocol for folate?

Folic acid is a water-soluble vitamin that is required for many metabolic processes and as such a patient with a subnormal serum folate concentration may benefit from oral supplementation with folate. However, the effects of not supplementing such patients with folic acid are not as clear as they are for cobalamin. Also, the exact amount required for supplementation is not clear. We currently suggest supplementing cats or small dogs with 200 µg orally once a day and larger dogs with 400 µg once a day for one month. Folate or folic acid for oral supplementation is available from any health food store or from the vitamin section of any grocery - or department store. Over-supplementation is not a concern as excess folate is excreted in the urine.

Effect of drugs on gastrointestinal function testing?

We frequently get asked whether any number of drugs have an effect on the measurement of serum concentrations of cobalamin, folate, TLI, and/or PLI concentrations. The short answer is – "no" for any of them. We are not aware of any direct interferences of any drug on these serum parameters. Thus, the only mechanism by which a drug may alter these parameters is by improving the disease process, leading to normalization of the parameter. However, as long as clinical signs are present this would not be a likely scenario. Also, it should be noted that supplementation of cobalamin leads to decreases in serum folate concentrations. We believe that such decreases in serum folate concentrations are clinically meaningful and suggest supplementation with folate (see above) if this should occur.

Heterobilharzia americana (Schistosomiasis) in dogs

If you have identified *Heterobilharzia americana* in one of your canine patients, it may have been a surprise diagnosis based on a biopsy specimen or necropsy. In a retrospective case series, we found that the majority of cases diagnosed by examination of a biopsy specimen or by necropsy had not been suspected to be infected by the clinician. In fact, the only cases where the veterinarians reported *H. americana* as a differential diagnosis in the history portion of the request form were when that dog or another dog in the household had been previously diagnosed with Schistosomiasis. So why is this parasite not widely suspected to be a cause of disease in dogs by the profession?

H. americana is a trematode belonging to the Schistosomatidae family and infects dogs, but has also been reported in a wide range of other mammalian species, including raccoons, nutria, wolves, coyotes, foxes, white tail deer, bobcats, mountain lions, and horses. A larva (miracidium) hatches from the egg upon contact with water and penetrates a freshwater snail (i.e., *Lymnaea cubensis* or *Pseudosuccinea columella*). Asexual reproduction occurs within the snail and multiple fork-tailed larvae (i.e., cercariae) are released into the water and penetrate the skin of the mammalian definitive host. The adults develop in the liver and then migrate to the mesenteric vessels to mate and reproduce. The eggs then migrate into the intestinal lumen and are excreted in the feces. Often the eggs are diverted into the portal circulation and may be distributed into various tissues rather than completing their migration through the gut. These eggs stimulate an inflammatory reaction by the host contributing to a variety of systemic clinical symptoms.

The prevalence of this parasite is lower compared to that of parasites transmitted through contact with soil because contact with infected fresh water is essential for transmission. The distribution of *H. americana* is also limited to the south Atlantic and Gulf Coast regions. The potential for geographic translocation exists and has previously been reported when infected raccoons were imported to Kansas from Texas, leading to an endemic appearance of *Heterobilharzia americana* in Kansas. A similar situation has been observed when *Schistosoma mansoni,* a related parasite of humans, was translocated to Brazil through the slave trade. A suitable intermediate host snail was present in Brazil establishing this parasite, which today is still a major public health concern in Brazil. If the suitable intermediate host were to be widely distributed in the United States, *H. americana* has the potential to extend its geographic presence, thus suggesting that this parasite may become a significant problem in veterinary medicine throughout the USA.

Clinical signs

The clinical presentation of Schistosomiasis in dogs is variable. From our retrospective case series of 238 canine cases, reported clinical signs (from most to least common) were diarrhea, weight loss, anorexia or hyporexia, vomiting, hematochezia, lethargy, and/or polyuria/polydipsia. Ultimately, clinical signs are due to granulomatous inflammation and fibrosis secondary to entrapped eggs in the gut or in other organs. Eggs were found (in descending order) in the small intestine, liver, large intestine, pancreas, lymph node, lung, spleen, and stomach. Severe cases that lead to death or euthanasia occurred in dogs with end stage liver failure or in hypercalcemic dogs with renal failure. Increased parathyroid hormone-related protein (PTHrP) levels have been documented in dogs infected with H. americana but no evidence of neoplastic disease, complicating the diagnosis of this disease further.

Diagnosis

The diagnostic tests for *H. americana* currently available are fecal sodium chloride sedimentation and fecal PCR. Fecal sedimentation is performed with sodium chloride because *H. americana* eggs will hatch in tap water. Five to 10 grams of feces are washed with saline in a graduated cylinder every hour until the supernatant is clear. Several aliquots of the sediment are then viewed under a light microscope for the detection of the eggs. This test is not typically performed in clinical practice. The fecal PCR test, performed at the GI Lab, amplifies a section of the 18S ribosomal DNA gene of *H. americana*. The average sensitivity of this assay is 1.5 eggs per gram of feces and the turnaround time is 1-2 business days. Both of these tests rely on fecal egg shedding, which may be intermittent. For this reason, submitting multiple stool samples from different days is optimal. *H. americana* eggs have also been detected by fecal floatation and by a direct fecal smear; however, this is uncommon and these tests should not used to rule out an infection. Dogs have also been diagnosed by biopsy of various organs including endoscopic biopsies of the small and large intestine. PCR can also be performed on biopsy samples.

Heterobilharzia americana (Schistosomiasis) in dogs (continued)

Treatment

Reported treatment for *Heterobilharzia americana* includes fenbendazole at a dosage of 40 mg/kg every 24 hours for 10 days and praziquantel at a dosage of 25 mg/kg every 8 hours for 2 days. Oral tablets or subcutaneous injections of praziquantel have been administered based on the available formulation of the drug and whether or not the patient was vomiting. One patient was successfully treated with fenbendazole at 24 mg/kg every 24 hours for 7 days and praziquantel at 10 mg/kg every 8 hours for 2 days. A single dose of praziquantel at the standard cestode deworming dose (5 mg/kg) was administered to a dog with Schistosomiasis and that treatment was shown later on to have failed. This dog was then treated successfully with a single subcutaneous dose of 11.3 mg/kg of praziquantel concurrently with an oral dose of praziquantel at 30 mg/kg. If patients are diagnosed and treated early during the disease, the prognosis is good. In addition to the 50 mg tablets licensed for treatment of tapeworm infections in dogs (Droncit; Bayer) praziquantel is also available as a 600 mg tablet licensed for the treatment of schistosomiasis in humans (Biltricide; Bayer).

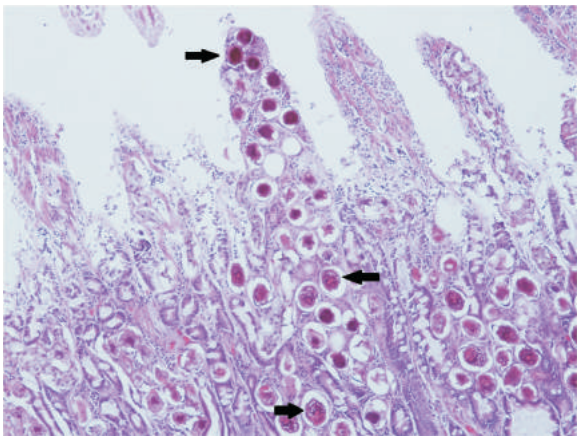


Figure 1: This image from a dog with Schistosomiasis shows small intestinal villi with migrating *Heterobilharzia americana* eggs (see arrows).

PATIENT SAMPLES ARE SOLICITED FOR A COMPARATIVE DIAGNOSTIC METHODS STUDY:

We are currently investigating a serologic antigen test that has been shown to successfully detect several schistosome species in humans and other mammalian species. However, the clinical usefulness for the diagnosis of *H. americana* in dogs has not previously been evaluated.

Eligible patients:

1) dogs with a clinical suspicion of *Heterobilharzia americana* (gastrointestinal signs, hypercalcemia, elevated liver enzyme activities, etc.)

2) dogs in a household with other infected dog(s)

3) dogs previously diagnosed with *H. americana* after treatment

Sample submission:

Please contact us prior to sample submission as all owners will need to sign an informed client-consent form before enrollment of the dog. At least 5 grams of fecal material (10 grams ideally) and a serum sample are required for submission from each dog. The feces will be used to perform a fecal sedimentation and a fecal PCR. The serum sample will be used to perform the antigen test. The fecal PCR test will be performed by the Gastrointestinal Laboratory at the standard charge and with a standard turnaround time. For patients enrolled in the study, the fecal sedimentation test, the serum antigen test, and a second fecal PCR test will be performed at no charge at the Parasitology Laboratory with a turnaround time ranging from 2-7 days. If you are interested in participating in this study please contact Jessica Rodriguez, DVM at or Karen Snowden, DVM, PhD, DACVM (project supervisor) at 979 862-4999.

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We would love to hear from you!