# Update in Feline GI Syndromes

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This presentation will look at newer information regarding Inflammatory Bowel Diseases (IBD), alimentary lymphomas, triaditis, cholecystitis, Tritrichomonas foetus and utilization of rectal cytology.

**Inflammatory bowel disease**

Inflammatory bowel diseases are a diverse group of intestinal disorders, which have been grouped together based on their histopathological commonalties. Another term that is used and is a bit more descriptive is lymphocytic-plasmacytic enteritis (LPE). This is somewhat unfortunate, in that the tendency is to see "IBD" as a diagnostic endpoint, with a sole treatment protocol, rather than as a description of a pathophysiologic response. Ideally, the term “IBD” should be restricted to those forms in which all identifiable etiologies have been ruled out, thereby restricting use of the term to “idiopathic” causes.

For normal intestinal immunity, an intact mucosal barrier is required. Because antibodies to enteric antigens are found in some people with IBD, a defect in mucosal permeability is suspected in the human condition. It is unclear whether increased gut permeability is a cause or a result of inflammation; likely it is a combination of both. Based on the cytokine expression, it appears that immune dysregulation plays a role in feline IBD and that IBD in cats has a complicated pathogenesis with both pro-inflammatory and immunoregulatory features.

Interesting work has also implicated bacteria in IBD, however their role may be protective rather than pathogenic. In intestinal biopsies from cats with clinical signs and hisitology compatible with IBD, Simpson found:

- increased numbers of mucosa-associated Enterobacteraceae, based on fluorescent in situ hybridization (FISH technique) with probes for 16s rDNA;
- that the total numbers of mucosal bacteria strongly correlate with changes in mucosal architecture (namely villous atrophy and fusion), density of cellular infiltrates (esp. macrophages). In addition to Enterobacteraceae, invasive Clostridium spp and E. coli were found;
- up-regulation of cytokines (esp. IL-8).

Other groups have studied the relationship between mucoal bacteria, histopathologic changes, cytokine responses, bacterial populations, and immune cell types, in cats with clinical disease. The concepts evolving from these studies may result in useful therapeutics and more specific and less invasive diagnostics.

Histologic considerations: The presence of increased inflammatory cells in an intestinal biopsy does not necessarily confirm a presumptive diagnosis of IBD. One must eliminate the other known and detectable causes of chronic inflammation first. Therefore, parasitic infestations and retroviral infections should be tested for. A dietary trial with a limited antigen diet should be undertaken for a minimum of 6-8 weeks. And the possibilities of bacterial overgrowth or lymphoma should be considered.

**Neoplastic transformation**

There is evidence that LPE/IBD originally diagnosed by biopsy may become (histologically verified) diffuse intestinal lymphoma after years of clinically being controlled with diet, metronidazole and prednisolone. It is possible that severe lymphocytic-plasmacytic IBD may be a pre cancerous lesion. It is also noteworthy, however, that partial thickness endoscopic biopsies may miss the telltale neoplastic lymphocytes due to their preferential localization in the muscularis and deeper layers. In addition it has been shown that both conditions may co-exist in a patchy distribution. By harvesting 9-12 good quality endoscopically collected tissue samples from each section of the gastrointestinal tract, this problem may be minimized. It has been recommended that full thickness biopsy specimens of the jejunum and ileum be obtained either by laparotomy or laparoscopy for accurate diagnosis as endoscopic biopsy specimens may not be adequate for differentiating between IBD and lymphosarcoma. Logistical limitations of access play a role.

Low-grade (lymphocytic) alimentary lymphoma (LGAL) is a recently described entity displaying many microscopical features similar to lymphoplasmacytic enteritis (LPE). Evaluating 53 cases of these conditions, Briscoe reviewed the histopathological and immunohistochemical features of LPE and LGAL to determine if specific features are useful in distinguishing between these disorders.

There were 24 cases of LPE, 12 were mild, seven were moderate and five were marked in severity. The ileum and jejunum were the most common sites affected for both LGAL and LPE (70-90% of cases). Involvement of the stomach was more common with LPE (29%) than LGAL (7%). Complicating matters further, 12 cases of LGAL (41%) had evidence of concurrent LPE.

Microscopic features significantly associated with LGAL were epitheliotropism, involvement of the muscularis propria and/or serosa, more severe infiltration and more severe changes to the villus and crypt architecture. In contrast, plasma cell infiltration within the mucosa was a feature of LPE.

**Optimizing endoscopy (technique)**

After examining the stomach and proximal duodenum, remove the endoscope and prepare for exploratory laparotomy. After evaluating the abdominal organs and peritoneal cavity, make an incision in the proximal duodenum circumscribed by a purse-string suture. Insert the endoscope and slide the bowel up over the endoscope. While visualizing the mucosal surface of the intestine, place stay sutures at spots that you think should be biopsied. Withdraw the scope, close the purse-string suture and take full thickness biopsies of those suspicious areas as well areas that look abnormal from the serosal aspect of the bowel.

**Gastrointestinal lymphoma**

Lymphoma is the most common form of gastrointestinal (GI) neoplasia in cats. Alimentary lymphoma has, in the past, been given a poor/grave prognosis in most of the literature. Richter differentiated between lymphocytic and lymphoblastic lymphomas in a group of 67 cats by taking extensive endoscopic biopsies. He found that 90% of the small cell (lymphocytic) form involved the jejunum and ileum and might have been missed with endoscopy. In contrast, 50% of cases of lymphoblastic, large cell lymphoma, involved small bowel only; the remainder involved stomach or stomach and small bowel. Histologically, 25% of the 67 cases had lymphoblastic lymphoma, 75% had lymphocytic.

Low-grade alimentary lymphoma (LGAL) was diagnosed by histological and immunohistochemical evaluation of full-thickness biopsies from multiple regions of the gastrointestinal tract collected during exploratory laparotomy in 17 cats. (Lingard) The most common ultrasonographic finding was normal or increased intestinal wall thickness with preservation of layering. Ultrasound-guided fine-needle aspirates of mesenteric lymph nodes (n=9) were incorrectly identified as benign lymphoid hyperplasia in eight cats, in which the histological diagnosis from biopsies was lymphoma. There was neoplastic infiltration of more than one anatomic region of the gastrointestinal tract in 16/17 cats. The jejunum (15/15 cats) and ileum (13/14 cats) were the most frequently affected sites, followed by the duodenum (10/12 cats).

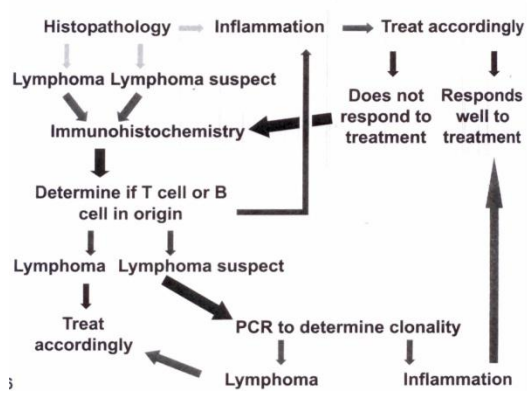
Numerous studies have shown that treating lymphocytic lymphoma using prednisolone and clorambucil is effective and minimally stressful, (i.e., no clinic visits are required). There is no benefit to outcome using modified Madison-Wisconsin multiagent protocol. The median disease free interval has been reported to be 20.5-26 months (range 5.8-49 months). Should rescue be needed, cyclophosphamide is used with generally excellent results (Richter, Stein). Cats with lymphoblastic lymphoma responded poorly to chemotherapy using either CVP (cyclophosphamide, vincristine, prednisolone) or ACOPA (CVP + doxorubricin and L-asparaginase). Cats with lymphoblastic lymphoma are more likely to experience recurrences of abdominal masses.

Treatment of lymphoma is most effective when tailored to the specific cell type, hence numerous studies have been performed to attempt to determine the best ways to identify the cells. Pohlman examined 50 cases of feline gastrointestinal lymphoma. Tissue sections were stained with HE, phosphotungstic acid hematoxylin, and immunohistochemical stains (anti-CD3, anti-CD79a, and anti-BLA.36). Small intestinal lymphoma was the most common form found, with 74% of cats affected: T-cell tumors comprised 52%; 38% were B-cell tumors. Gastric tumors were diagnosed in 24% and 18% were present only in the stomach. All gastric lymphomas were of B-cell lineage. Of the 8 cats (16%) that had lymphoma of the large intestine, 88% had B-cell tumors and 12% had T-cell tumors.

The strongest association between gastrointestinal lymphoma immunophenotype, histologic classification, and location occurred in the stomach, where there was a predominance of diffuse large B-cell lymphoma of immunoblastic nuclear type.

Evaluating surgical and endoscopic intestinal biopsy specimens from 63 cats with a history of chronic diarrhea or vomiting or weight loss, Kiupel developed a diagnostic algorithm that begins with histologic assessment, followed by immunophenotyping and then applies PCR to determine the clonality of the lymphocytes, to more accurately differentiate between intestinal lymphoma and inflammatory bowel disease (IBD). A diagnosis of lymphoma or inflammation was based on microscopic examination of hematoxylin and eosin (HE)-stained sections alone, HE-stained sections plus results of immunohistochemical labeling (IHC) for CD3e and CD79a, and HE staining, immunophenotyping, and polymerase chain reaction (PCR) results for B and/or T cell clonality.

Important histologic features that differentiated intestinal lymphoma from IBD included lymphoid infiltration of the intestinal wall beyond the mucosa, epitheliotropism (especially intraepithelial nests and plaques), heterogeneity, and lymphocyte nuclear size.




Tests for clonality: PARR assay is a PCR assay in which DNA is being amplified. The results determine whether the majority of cells in the sample are derived from the same original clone (most consistent with neoplasia), or from multiple clones (most consistent with a reactive process). The limitations of PARR in feline lymphoma are a low sensitivity, i.e., there are approximately 35% false negatives. The tests complement each other and both should be run together. Both tests can be run on blood; PARR can be run on stained slides but not on formalin fixed, paraffin embedded samples or cover-slipped slides.

With flow cytometry live cells are stained with labeled antibodies that bind to proteins expressed on the cell surface. Different types of lymphocytes express different protein (for example T cells express the protein CD3, and B cells express the protein CD21). The cells are analyzed on a flow cytometer, which tells us how many cells of each type are present. This information allows us to determine the lineage of the cells present, and whether they are homogeneous (more consistent with neoplasia) or heterogeneous (more consistent with a reactive process). (www.cvmbs.colostate.edu/ns/departments/mip/cilab/which_test.aspx)

The Bcl-2 gene is a member of the rapidly expanding Bcl-2 family of genes that regulate apoptosis. Bcl-2 has been shown to repress cell death triggered by a diverse array of stimuli, including chemotherapy and gamma irradiation. Kano et al confirmed the expression of Bcl-2 in T-cell lymphoma cell lines using an immunoblot assay. Their conclusion was that pending further evaluation, Bcl-2 expression might be useful in the differential diagnosis of feline tumors.

**Triaditis *vs.* "IBD"**

The clinical signs of IBD vary with location of the inflammatory process: duodenal and gastric lesions usually present as vomiting and weight loss while small intestinal or colonic lesions present as diarrhea +/- weight loss (if small intestinal). However, some colonic IBD may cause vomiting as well. There are also cats in whom the inflammatory process extends beyond the gastrointestinal tract and affects the liver, biliary tree and pancreas. This is fondly termed "triaditis". These cats may present with signs attributable to these organs, which may or may not include vomiting and diarrhea.

Anatomically and pathophysiologically, it is "logical" to understand why this may occur. In approximately 80% of cats, the accessory pancreatic duct is absent. The pancreatic duct enters the common bile duct before the latter opens into the duodenum at the major duodenal papilla. If there is pathology in the distal common bile duct, either ascending from the duodenum or originating in the duct itself, (such as infection or cholelithiasis), this could predispose to pancreatitis because of the functional relationship between the major pancreatic and common bile duct sphincters in the cat. Experimentally, it has been shown that when the major pancreatic duct is perfused with bile acids, marked structural changes occur not only within the pancreatic duct, but also in the pancreas itself. This is why feline pancreatic disease is a common cause of extrahepatic biliary obstruction.

**Cholecystitis**

Cholecystitis generally presents as a vague malaise with inappetance and dehydration, (pretty much like everything else in the cat). Vomiting may be present. While this too may involve a lymphocytic/plasmacytic inflammatory infiltrate of the gall bladder wall, it more often is suppurative. If surgical evaluation occurs, and the gall bladder looks inflamed or if it does not compress and empty normally, or if ultrasound findings are suggestive of cholecystitis, bile aspiration and culture (aerobic and anaerobic) should be performed. This can be done safely using ultrasound guidance and aspirating transhepatically.

**Tritrichomonas foetus**

Tritrichomonas foetus has recently been recognized as a common cause for recurrent large bowel diarrhea in North America. In a study of 117 cats at a cat show, the prevalence of T. foetus was 31%, of Giardia lambia, 31% and of coinfections with both protozoal parasites, 12%. The conclusion was that T. foetus infected catteries are common. There is no evidence to show transmission of T. foetus by water, food or contact with other species. In contrast, Giardia sp. infection is significantly associated with source of water and direct contact with other indoor-outdoor species. Most cats experience spontaneous resolution of diarrhea within 2 years (with a

median of six months) after the diagnosis, despite persistence of infection on the basis of fecal PCR. Changes in diet, administration of medication or other stressors result in recurrences of diarrhea in approximately 50% of the cats. By 2-5 years after diagnosis, infection was no longer detectable in 50% of the cats by PCR so the conclusion was made that the long-term prognosis for resolution and cure is good for cats infected with T. foetus. Since 2006, reports on the prevalence of T. foetus infection in cats with diarrhea have come out of the United Kingdom, Switzerland, Australia and Greece showing a worldwide distribution.

T. foetus may be identified with light microscopy as a motile flagellated organism, which looks similar to Giardia. A commercially available test, (InPouch Feline TF®) has been validated as an excellent way to specifically identify this organism and is sensitive and specific for detection of T. foetus in feline feces. Inoculate a pouch with 0.025-0.05g of fresh feces (peppercorn or grain of rice size), then incubate it standing upright, at room temperature (optimally 25C), in the dark and examine the pouch for trophozoites at 20X every other day for 12 days. Approximately 50% of infected samples tested will be positive in three days. Light microscopy (on either a wet mount or rectal cytology), InPouch culture (or modified Diamond culture in commercial laboratory) may result in false negative results. A third type of test, PCR for Tritrichomonas may be submitted to the appropriate facility. It is more sensitive and exquisitely specific and is the gold standard; it may also occasionally fail to detect the organism in an affected cat.

Ronidazole is used for the treatment and elimination of T foetus in cats at 30 mg/kg PO SID. Higher doses are not more effective and at higher doses, there is a risk of neurotoxicity.

**Rectal cytology**

For large bowel diarrhea, cytology from rectal scrapings and gram stain of prepared slides may be very helpful in achieving a definitive diagnosis in many cases of large bowel diarrhea. This test harvests cells and organisms from the lumen-colon wall interface. Insert a moistened sterile culture swab 2-3 cm into the rectum of the cat and rotate it gently. Roll this swab gently and thoroughly on two microscope slides and store the swab in the culturette medium. Submit the slides for cytology plus gram stain and follow with the swab as indicated by the cytology results. Rectal cytology may diagnose bacterial or non-septic suppurative colitis, Cryptosporidium, Giardia, Tritrichomonas, Campylobacter infections as well as fungal hyphae. The presence of clostridial spores must be interpreted carefully and a fecal enterotoxin assay should be performed to determine if disease-causing clostridial enterotoxin is present or not.

**References available upon request**