Canine parvovirus-2 (CPV-2) causes severe enteric disease, mainly in young susceptible dogs. The virus initially replicates in lymphoid tissue and reaches the small intestine as a result of viremia. Destruction of replicating cells in small intestinal crypts (Fig. 1) leads to the characteristic clinical signs.

A few years after its emergence in the late 1970s, CPV-2 was replaced by two antigenic variants, CPV-2a and CPV-2b. Those two types are now distributed worldwide.

A third antigenic variant, designated CPV-2c, was first reported in Italy in 2001 and has more recently been detected in the US. Genetically, CPV-2c variants differ from the CPV-2b strain by an amino acid substitution at residue 426 of the capsid protein. The amino glutamic acid has replaced aspartic acid in the CPV-2c strains. This residue is located in a region of the major capsid protein that is considered to play a role in immunogenicity.

Our standard methods to diagnose CPV-2 infections in tissue samples consist of direct fluorescent antibody staining of frozen sections of small intestine (Fig. 2) or immunohistochemistry on paraffin embedded tissue (Fig. 3). For antemortem diagnosis we use real-time PCR on fecal samples or fecal swabs (Fig. 4). These methods diagnose CPV-2 but do not differentiate between strains. One of the applications for strain differentiation is the determination of the incidence of CPV-2c in an area and its correlation with severity of the disease. It is certainly true that CPV-2c can induce severe lesions in susceptible dogs. In the recently reported American CPV-2c cases, many dogs had either mucoid yellow or hemorrhagic diarrhea. In Italy, CPV-2c has been responsible for mucoid diarrhea, leukopenia, and lymphopenia in puppies. It is probably not correct, however, to assume that CPV-2c strains are by definition more virulent than CPV-2a or CPV-2b strains.

Another situation in which strain differentiation is valuable is the occurrence of parvovirus signs in a recently vaccinated dog. It has been reported that it is not uncommon to find both the vaccine virus and the field strain the dog was exposed to around the time of vaccination in the same fecal sample.

Recent papers have shown that the active immune response resulting from vaccination with current vaccines cross protects against CPV-2c, although these vaccines do not contain CPV-2c at this point. The situation may not be so clear-cut for passive immunity. It is feasible that the minimum level of passive antibody needed to protect against CPV-2c field strains is higher than for field strains that are homologous to those present in the vaccine.

In order to be able to monitor the incidence of CPV-2c and to provide comprehensive strain differentiation in situations such as 1) severe parvovirus disease in well vaccinated dogs, 2) outbreaks or endemic infections in shelters, or 3) parvo-like symptoms in recently vaccinated dogs or in young puppies from well vaccinated females, we recently introduced a new molecular parvovirus test combination, consisting of PCR followed by sequencing to determine the strain type. The preferred samples are fecal samples, fecal swabs or small intestine. Our fee for this service is $100 and the turnaround time is 7-10 days.
EIA Testing Streamlined through GlobalVetLink Services

For veterinarians who have access to the internet, online EIA certificates and results can save time, postage, and administrative costs. The Michigan Department of Agriculture has worked with GlobalVetLink to make GVL’s electronic certificate of EIA testing available to Michigan accredited veterinary practitioners. Best of all, electronic EIA certificates are accepted by all state veterinarians’ offices.

Michigan equine veterinarians have a choice of laboratories to which they can send their specimens for testing. DCPAH has joined the list of available labs and offers EIA testing through GVL at a discounted price! Regular EIA submissions using the 7-part federal form are $10 each. GVL-submitted requests are only $8 each. DCPAH still offers same-day turnaround on specimens received by noon. Certificates are available for printing by participating veterinarians within minutes of results being released.

Facts about GlobalVetLink’s services, which are fee based, can be viewed at http://www.globalvetlink.com. Assistance is available from GlobalVetLink representatives at 515-296-4033. We recommend that you sign up with GVL now to get ready for EIA testing this spring.

Bone Marrow Samples: Cutting to the Core of the Problem

By: DCPAH Clinical Pathologists

Complete blood counts (CBCs) are screening profiles that may reveal pathologic problems (e.g., thrombocytopenia), processes (e.g., inflammation), or specific diagnoses (e.g., immune-mediated hemolytic anemia). However, the CBC is a single snapshot of hematopoiesis, providing a frozen image that reflects just the momentary balance of factors affecting circulating cell concentrations and appearances. When the CBC suggests altered cell production, or when laboratory or clinical findings suggest other problems within the hematopoietic factory, bone marrow evaluation may be helpful.

The most frequent indications for bone marrow examination are persistent and unexplained cytopenias, particularly moderate to severe nonregenerative anemia, but also neutropenia, thrombocytopenia, bicytopenia, and pancytopenia. In these conditions, bone marrow examination may yield nonspecific findings, such as hypoplasia or aplasia, or evidence of more specific abnormalities including immune-mediated cell destruction, hemic or nonhemic neoplasia, infection, or myelofibrosis. Bone marrow examination also may be indicated by atypical cells in blood, unexplained hypergublenemia, or hypercalcemia. It may be done to stage neoplasia, to investigate unexplained leukocytosis, thrombocytosis, or erythrocytosis, or to pursue occult infectious diseases.

Bone marrow samples are collected from sites expected to have active hematopoietic tissue, usually the iliac crest or proximal humerus in dogs and cats. Cytologic or histologic biopsies may be done, the former providing much better cell detail, the latter allowing thorough assessment of architecture, and the combination yielding the most information. In most cases, a CBC should be done the same day because CBC results can change quickly.

Bone marrow aspiration and core biopsy samples may be collected from the same bone, separating the sites enough that the first collection does not adversely affect the second. For aspiration, insert a Rosenthal or Illinois biopsy needle, remove the stylet, and aspirate bone marrow into the needle by forceful negative pressure with a 10 or 12 cc syringe. Limit hemodilution by terminating aspiration as soon as blood is seen entering the syringe. Within seconds, place small drops containing marrow particles onto slides and spread the material by using a gentle wedge or squash smearing technique (see Fig.1). For core samples, insert a Jamshidi needle through the cortical bone, remove the stylet, advance the needle 1-2 inches with a forceful twisting motion, and rotate the needle 360° several times before withdrawing it. Push the core sample out the top (not narrower tip) of the needle with the stylet, and gently place the sample into 10% buffered neutral formalin. Avoid dull needles; they hamper collection and decrease sample quality.

Submit aspirate smears (some unstained) and fixed core samples to DCPAH with CBC results and/or a blood smear. Keep the formalin container and unstained slides sealed in separate zip-locked bags to prevent formalin fumes from reaching the slides (adversely alters staining). Include relevant historical and physical examination findings, and the reason for bone marrow evaluation.

FIGURE 1: Stained bone marrow aspirate smears. The top smear is desirable because it is in the center of the slide and has many marrow particles (blue) with little blood. In contrast, the bottom smear extends off the slide and has too few particles and too much blood.
Diabetes mellitus (DM) is a chronic endocrine disorder with increasing incidence in cats, and the majority of cats have type II DM characterized by insulin resistance (as do humans). Cats that are overweight or greater than 10 years of age are at an increased risk of developing DM. Most naturally occurring fatty acids are in the cis-configuration. Some trans-fatty acids (TFA) are found naturally in meat (beef, pork, lamb) and dairy products, due to microbial hydrogenation of cis-unsaturated fatty acids in the rumen or intestines. The average TFA in ruminant fat is 5-8 g TFA/100 g fat. However, high levels of TFA are created during the hydrogenation and deodorization of oils used in manufacturing. The average TFA in hydrogenated oil is 45 g TFA/100 g oil. In humans, TFA intake is positively associated with the incidence of DM, and the risk of DM increases by 39% with a high TFA intake. The consumption of only 1 percent energy (%E) from TFA results in an increase in insulin resistance. For these reasons, the FDA mandated that starting in 2006, TFA content would be itemized separately in the Nutrition Facts on food labels, and the American Heart Association recommends that humans should consume no more than 2 g TFA/day based on a 2000 calorie daily diet (1 %E from TFA).

Exposure to dietary TFA may also increase insulin resistance and type II DM in cats, as it does in humans. The objectives of the study were to determine the TFA content and %E from TFA in commercial feline diets, and to determine if there was a correlation of dietary TFA to serum insulin in cats at increased risk of DM.

The TFA content was determined in 77 major brand cat foods, including those diets recommended for ‘at-risk’ groups (senior diets, weight control diets, adult diets) (Fig.1 [a and b]). Of the feline diets tested, 23% contained a level of TFA known to contribute to insulin resistance in other species (>1 %E).

Cats were identified in the following groups: NORMAL, less than 10 years of age with body condition score (BCS) of 4-6 (n=27); FAT, less than 10 years of age with BCS of 7-9 (n=23); and SENIOR, 10 years of age or older with BCS of 4-6 (n=6). All cats were determined to be healthy based on clinical history and physical examination, and cats were excluded if there was any history of other underlying disease. Serum insulin, glucose, and TFA concentration was determined for each cat. In addition, complete diet histories were obtained, and a sample of each cat’s diet was obtained for TFA analysis. FAT cats had significantly higher BCS, body weight, and % body fat as compared to NORMAL cats and SENIOR cats. FAT cats had a significantly higher concentration of serum insulin and serum insulin:glucose (I:G) ratio as compared to NORMAL cats or SENIOR cats (Fig.2). Glucose concentrations did not significantly differ among groups. There were no significant differences in serum TFA concentrations, dietary TFA concentrations, or dietary %E derived from TFA among groups. Insulin concentration and I:G were significantly correlated to BCS, body weight, and % body fat, but not to serum TFA, dietary TFA, or dietary %E from TFA. Cats with higher risk of DM did not show elevated serum TFA or dietary intake of TFA. However, there was a significant difference in dietary %TFA to serum %TFA ratio, with NORMAL cats having a higher ratio as compared to FAT and SENIOR cats. While there does not appear to be a direct correlation of dietary TFA to insulin concentration, the significantly higher dietary TFA to serum TFA ratio in NORMAL cats may imply metabolic or absorptive differences in TFA handling compared to FAT or SENIOR cats. TFA could still contribute to the development of insulin resistance and DM in predisposed individuals.

This project was supported in part by the Winn Feline Foundation.
DCPAH has a new logo, a new look, and the same GREAT service!

Watch for our NEW look and newsletter design in the New Year—2009!

Comments and Suggestions to:
Michelle Kryska, kryskam@dcpah.msu.edu