

Diagnostic Services Laboratory, Atlantic Veterinary College
 University of Prince Edward Island
 550 University Avenue, Charlottetown PE, C1A 4P3
 Phone: 902.566.0860 • Fax: 902.566.0723 • www.upei.ca/diagserv/

Diagnostic Update



Serologic Testing for Lyme Disease in Dogs

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By Barbara Horney, Veterinary Clinical Pathologist

Lyme disease is associated with *Borrelia burgdorferi* (*Bb*) infection, usually through a tick bite, and can be considered to be an emerging disease in the Atlantic Canada region. The *Ixodes scapularis* ticks that carry this agent appear in our region either by expansion of their geographic range or by being carried from endemic areas on migrating birds. In general, the proportion of dogs showing serologic evidence of exposure to *Bb* in our region is low (less than 5%).

The following information and recommendations on testing for Lyme disease have been adapted from the **2006 ACVIM Small Animal Consensus Statement on Lyme Disease in Dogs.**¹

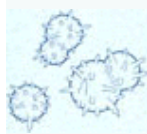
In endemic areas, 95% of dogs exposed to *Bb* remain asymptomatic. The clinical signs of Lyme disease are relatively nonspecific and may be similar to those associated with other infectious and noninfectious agents. The consensus statement maintains that diagnosis of canine Lyme disease cannot be made from an individual test and that presumptive diagnosis of Lyme disease should include:

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1. Evidence of exposure to *Bb* (usually by demonstration of serum antibodies to *Bb*),
2. Clinical findings consistent with Lyme disease which include: fever, arthropathy (lameness), nephropathy (proteinuria),
3. Consideration of other differentials, and
4. Response to treatment.

Types of tests for antibody to *Bb* available:
ELISA and IFA tests: Identify serum antibodies to



Borrelia but require Western blot testing to help differentiate between a true positive and a vaccine response or infection with other *Borrelia* species.

IDEXX SNAP-4Dx (3Dx): Includes a qualitative test for antibody to the C6 antigen (expressed when a dog is infected with *Bb* and not present in the vaccine). A positive test indicates exposure to the *Bb* organism but does not prove clinical disease. The antibody response to the Lyme vaccine should not be detected as a positive result by this method. A quantitative C6 antibody test is also offered through IDEXX which can be used to demonstrate decreasing antibody levels after therapy.

For patients with suspicious clinical signs: Either testing type is valid. A positive result can support (but does not confirm) a diagnosis of Lyme disease, as long as other possible causes of the clinical signs have been ruled out. Demonstration of rising titres (acute and “convalescent” samples) are not required as the clinical signs of Lyme disease usually develop after seroconversion. In seropositive dogs, the C6 quantitative test can be useful to identify decreasing antibody levels as a response to therapy, with serum samples taken before and 6 months post treatment. The consensus statement also recommends testing seropositive dogs for proteinuria which can be associated with Lyme nephropathy.

Testing healthy dogs:

This is somewhat controversial¹ but it is generally not recommended in areas with a low prevalence of exposure to *Bb*, as the proportion of false positives can be high even with a sensitive and specific test.²

Testing through the Atlantic Veterinary College Diagnostic Services (AVC DS):
When submitting a canine serum to test for antibodies to *Bb*, please specify which test you would like:

<u>Test</u>	<u>Sample required</u>	<u>Testing location</u>	<u>Cost (2008)</u>
IDEXX SNAP-4Dx^{†*}	1 ml serum	AVC	\$30.00
IFA[§]	1 ml serum	Michigan (Sent though AVC)	\$65.00

[†]*In cases in which Lyme disease is the most likely diagnosis and antibiotic therapy is instituted, a C6 quantitative test pre- and post- therapy may help to evaluate response to treatment. This test can be requested through AVC (forwarded to an IDEXX laboratory - please call AVC DS for cost)*

^{*} The 4Dx assay also includes tests for *Anaplasma phagocytophilum*, *Ehrlichia canis* and Heartworm.

[§]*Followup testing of an IFA positive result by Western blot analysis (forwarded to Cornell for analysis) is recommended to differentiate from response to a vaccine or other Borrelia species.*

References:

1. Littmen M, et al. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment and prevention. *J Vet Intern Med.* 2006;20:422-434.
2. Peregrine A, et al. Screening dogs in Ontario for *Borrelia burgdorferi* and *Ehrlichia canis* should be selective rather than routine. *Can Vet J.* 2007;48:673.

***Strongyloides stercoralis* infection in a puppy**

By Gary Conboy, Veterinary Parasitologist

A fecal sample was submitted for examination from a 3.5 month-old, male Yorkshire terrier with clinical signs of a cough and diarrhea. The dog had been purchased and transported from western Canada to Prince Edward Island (PEI) 1.5 months earlier. The dog had been treated with Interceptor (milbemycin oxime) for presumed lungworm infection due to a persistent cough that occurred

4 weeks after arriving on PEI. The cough improved with deworming. The puppy then developed diarrhea, was positive for *Giardia canis* cysts on fecal flotation and was treated with a 5 day course of fenbendazole at 50 mg/kg. After a short period of clinical improvement, the cough and diarrhea returned and progressively worsened. A second fecal sample was submitted for flotation and Baermann examination. The flotation was still positive for *Giardia canis* cysts and the Baermann detected first-stage larvae of *Strongyloides stercoralis* (Figures 1 & 2). The puppy was treated with ivermectin (0.2 mg/kg SQ) and showed marked improvement, with clinical signs resolving over the next 7 days. Post-treatment Baermann fecal examinations were negative.



Figure 1: Iodine stained first-stage larvae of *Strongyloides stercoralis* recovered by Baermann fecal examination from a puppy with clinical signs of a cough and diarrhea.

Strongyloides stercoralis infects the small intestine of dogs and cats and can cause a life threatening enteritis with clinical signs of diarrhea. Animals acquire infection by the ingestion or direct skin penetration of infective third-stage larvae. Larvae of *S. stercoralis* undergo migration through the lungs during their developmental cycle, and this was the cause of the cough in this patient. *Strongyloides stercoralis* occurs worldwide and it is not



Figure 2: Anterior-end of first-stage larvae of *Strongyloides stercoralis* larvae showing characteristic esophagus.

known whether the dog acquired the infection in western Canada or PEI. The prevalence of *Giardia* infection in puppies is high (up to 40%) and infection can result in diarrhea, but most infections are subclinical. The presence of *Giardia* in this dog was likely incidental. This is the second case of *S. stercoralis* we have diagnosed in a dog with clinical signs involving chronic cough. Fenbendazole and ivermectin have both been recommended for use in the treatment of dogs infected with this parasite. However, the 5 day course of fenbendazole given for the *Giardia* did not result in a cure. *Strongyloides stercoralis* is a serious pathogen in humans which heightens the importance of proper diagnosis, treatment and diagnostic post-treatment follow-up in cases involving dogs or cats.

The Therapeutic Drug Monitoring of Phenobarbital

By Sandra McConkey, Veterinary Clinical Pathologist and Pharmacologist

Therapeutic drug monitoring (TDM) is the measurement of the plasma or serum drug concentrations in patients to determine if the dosage is correct. It is typically used for drugs with a narrow therapeutic index or variable pharmacokinetics. Diagnostic Services at the

Atlantic Veterinary College does TDM for more than 150 clients every month. The majority of samples are evaluated for phenobarbital and potassium bromide (KBr) concentrations with lower numbers of samples submitted for digoxin, theophylline, gentamicin and cyclosporine. Uncommon drugs can be evaluated by special request.

Phenobarbital has both a narrow therapeutic index and a variable half-life. In fact, the therapeutic response to phenobarbital corresponds better with its serum concentration than its dosage. Most dogs require a phenobarbital concentration within a therapeutic range of 54-190 $\mu\text{mol/L}$ for sufficient seizure control. The wide range allows for several upward adjustments of the dosage over time if control is lost. The addition of a second anti-convulsant may be suggested at 120-140 $\mu\text{mol/L}$ if control is insufficient. It is best to wait until a drug concentration has reached steady state before initial testing. This takes five half-lives which is ~ 2 weeks for phenobarbital. After this, annual monitoring is recommended, as is reevaluation following any drug adjustment.

Peak samples are required if there is concern about toxicity whereas trough samples are used when concerned about efficacy. We suggest trough measurements at 8-12 hours post treatment for routine monitoring of phenobarbital. Occasionally evaluation of both peak (4-8 hours post-dosing) and trough samples are warranted. The combination allows us to calculate the half-life so that the dosing schedule can be individually customized to avoid potentially dangerous fluctuations. It is generally recommended to treat twice daily if the half-life of phenobarbital in your patient is greater than 30 hours and three times daily if it is less than 30 hours.

Phenobarbital treatment can be associated with several adverse effects, including sedation, polyuria, polydipsia, polyphagia and behavioral changes. Many animals experience one or more of these signs during the first few weeks of treatment, but these usually spontaneously resolve with the development of pharmacokinetic and pharmacodynamic tolerance. Occasional animals will continue to demonstrate these signs despite blood phenobarbital concentrations in the desired therapeutic range. These dogs should be changed to alternative anti-convulsants.

Mild elevations of ALT activity and mild to marked elevations of ALP activity can occur in animals on chronic phenobarbital therapy. These changes are believed to be due to phenobarbital induction rather than secondary to a toxic reaction. Phenobarbital-induced hepatic toxicity is typically associated with a greater than three-fold increase of ALT activity. There can also be a decrease in urea and/or albumin concentrations as well as increased bile acid concentrations in severe cases. Animals with phenobarbital-associated hepatotoxicity should be placed on anti-convulsants that are not metabolized by the liver or known to be hepatotoxic, such as KBr or levetiracetam.

When submitting samples for TDM, it is always best to include a full history of both the drug dosage and clinical response. This allows the clinical pharmacologist to provide informed comments. Occasional results are surprising when interpreted in view of the full history. They can reflect prescription or compounding errors, dietary changes, concurrent diseases or interactions with other drugs. Finally, never hesitate to call the laboratory if there are any questions about a testing procedure or if you wish to discuss an interesting case. We are here to help!

Mortality Continues in Maritime Finch Populations

By Scott McBurney and María Forzán, Veterinary Anatomic Pathologists, Canadian Cooperative Wildlife Health Centre, Atlantic Region

Since June of 2008, reports of sick and dying purple finches (*Carpodacus purpureus*) and American goldfinches (*Carduelis tristis*) have been circulating among those interested in bird watching and feeding in the Maritimes. Eye problems and emaciation were the most common conditions seen. In those finches submitted to diagnostic laboratories for post mortem examination, the causes of death were variable and included *Mycoplasma* conjunctivitis (1 incident), emaciation-starvation (3 incidents), drowning (1 incident) and predation (1 incident). However, similar to 2007, trichomoniasis, caused by *Trichomonas*, a protozoan parasite, was the predominant single cause of death identified in finches (8 incidents). The majority of birds affected by this disease were emaciated, likely because they were unable to ingest food as a result of throat, crop and esophageal lesions caused by trichomoniasis. Despite the fact that the lesions associated with trichomoniasis were quite remarkable microscopically, they were often very difficult to identify with the naked eye due to the small size of the birds. Therefore, it was important to do a thorough post mortem examination, including histology, before assuming the cause of emaciation in finches was simply starvation.

Mortality in the finch populations began much earlier this past summer compared to 2007, when dead finches were found in the late summer and early fall. The current seasonality is similar to that observed in the United Kingdom (UK), where trichomoniasis emerged as a significant cause of finch mortality in 2005. Climatic factors in the UK, particularly high precipitation, were initially thought to have played a role. This hypothesis has since been abandoned due to variability in the weather data.

Similar to the UK, a review of Maritime weather data for the last two summers did not identify consistent patterns related to precipitation and/or temperature. The other factor shared between the UK and Maritimes associated with trichomoniasis in finches was that most of the mortality was identified around feeding stations. This could simply reflect the fact that mortality is more easily observed in these locations by interested individuals. Alternatively, attracting and concentrating birds at specific locations may play a role in transmission of trichomoniasis. Also, because *Trichomonas* species are susceptible to desiccation, water can serve to promote survival of the organisms and higher densities of birds aggregating at limited water sources could increase disease transmission.¹ Therefore, water baths and drinking water sources provided by the public could be a part of the problem. However, at this early stage in the investigation of trichomoniasis in the Maritimes, much of the discussion remains speculative. More information is definitely required before we can clearly understand the epidemiologic factors that control the disease. Therefore, Canadian Cooperative Wildlife Health Centre, Atlantic Region (<http://atlantic.ccwhc.ca/>) strongly encourages the submission of any dead finches found within our region.

References:

1. Bunbury N, et al. *Trichomonas gallinae* in Mauritian columbids: implications for an endangered endemic. *J Wildl Dis.* 2007;43(3):399-407

Original article printed in the Canadian Cooperative Wildlife Health Centre Newsletter, Fall-Winter 2008-09, Volume 13, Number 3.

Work Safely: Packaging and Shipping of Samples

By Ellen McMahon, Veterinary Laboratory Technologist and Cora Gilroy, Veterinary Clinical Pathologist

Diagnostic Services places a strong emphasis on the health and safety of people involved in

packaging and shipping of samples from the time the sample is obtained until it is received.

We recently received a sample shipment in which formalin had leaked from a container. This not only posed a health risk to our staff, but also significantly delayed the processing of samples that day. Other samples risked being affected as exposure to formalin fumes can adversely affect slide staining.

There are many well documented adverse side effects to formalin exposure in humans, including irritation to the eyes, respiratory tract and skin. Skin exposure can lead to local redness, tingling and skin sensitization with the possibility of an allergic reaction with subsequent exposure. Symptoms involving the respiratory tract can include coughing, wheezing, burning pain of the nose and throat, shortness of breath and possibly pulmonary edema. Formalin is documented as a cancer causing agent, which is a concern with chronic exposure.

It is for these reasons, as well as to minimize the risk of zoonotic infections and to contain noxious odours, that it is paramount that proper packaging and shipping is adhered to. For ground transportation, the following packaging instructions are applicable:

1. Place the **sample** in a **waterproof primary receptacle (container)** such as a test tube with rubber stopper or a screw cap vial.
2. **Cushioning** must be provided between multiple receptacles and can include styrofoam tube holders or bubble wrap.
3. Place the **primary receptacle (with cushioning)** inside a **water tight secondary receptacle** (such as a zip lock bag) with an **absorbent material**. The absorbent material must be provided between the primary and secondary receptacles in sufficient

quantities to absorb all the liquid in the primary receptacle. One square of paper towel absorbs ~1 ml of fluid.

4. Place the **secondary receptacle(s)** in a sturdy **outside package** (Midland bag) with at least one surface having a minimum dimension of 4 inches.
5. Affix a label on the outside package indicating **“exempt animal specimen”**, **when appropriate**, as explained below (see Figure 1).

Figure 1: Sample Midland Courier Label

If a large piece of fixed tissue needs to be submitted, it can be placed in a large container of formalin for initial fixation of the tissue and then transferred to a smaller volume for shipping (as outlined on page 45 of the current Diagnostic Services Reference Guide). If gel-type ice packs are used, put them in a zip lock bag to contain moisture as they thaw and use cushioning material between the gel packs and the samples. **It is important to place the completed submission forms in a separate plastic bag.**

In February of 2008, an amendment was made to the Transportation of Dangerous Goods Regulations Section 1.42 indicating that the words **“exempt animal specimen”** must be marked on the package address label in order to

ship non-infectious specimens by courier. A modified excerpt from this amendment is as follows¹:

Professional judgment is required to determine if a specimen is exempt under this section. Factors such as the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions should be considered. Examples of specimens that may be transported under this section include

- *blood or urine specimens to monitor cholesterol levels, blood glucose levels, hormone levels, or organ function;*
- *biopsies to detect cancer; and*
- *specimens for antibody detection in animals.*

Bacteriology, virology, parasitology and histology specimens believed to contain viable micro-organisms must be packed in the same manner, but exemption 1.42 will not apply. Persons packaging these specimens must be trained in the transportation of dangerous goods for ground transport by Transport Canada.

If there are any questions concerning the status of a specimen for shipping, please contact the staff of Diagnostic Services. We are thankful that many samples are packaged and shipped in an appropriate manner. However, it is imperative that all remain vigilant so that hazardous materials are handled carefully to avoid the exposure of clinic, courier and laboratory staff to these substances.

References:

1. Regulations Amending the Transportation of Dangerous Goods Regulations. Canada Gazette Part II. Vol. 142, No. 4, February 20, 2008:131-304 (Registration SOR/2008-34)

Laboratory News

What's New in Diagnostic Services

By Linda Ruschkowski, Veterinary Laboratory Technologist

- Several new faces have appeared in Diagnostic Services. We heartfully welcome the following people to our fold: Dr. Elizabeth (Betsy) O'Neil (Clinical Pathology resident), Drs. Melanie Buote and Enrique Aburto (Anatomic Pathologists) and Darren MacEachern (Post Mortem leave of absence replacement).

We would also like to acknowledge the following permanent appointments of some of our staff members: Paulette MacDonald-Sheppard and Tammy MacDonald (administrative assistants in the Post Mortem office and the Virology laboratory), Sarah Bernard (Histology technologist), Jan Giles (Bacteriology technologist), Robin MacPhee (Virology technologist) and Nicole Murphy (Parasitology technologist).

We bid farewell to Dr. Basil Ikede who recently retired from his position as Department Chair (Pathology and Microbiology) and morphological pathologist.

We congratulate and wish all good luck in their endeavours!

- Diagnostic Services is looking forward to providing several continuing education sessions at the 2009 Atlantic Provinces Veterinary Conference. We hope to see you there!

Staff Focus

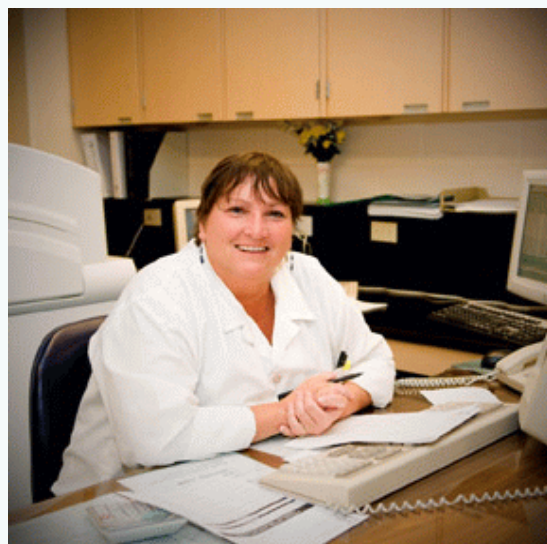
Ellen McMahon

By Shelley Burton, Veterinary Clinical Pathologist

Many will recognize the cheerful voice and prompt professional service offered over the last 20 years by Ellen McMahon, a technologist in the clinical pathology laboratory within Diagnostic Services. In addition to her skills in biochemistry analysis, urinalysis and protein electrophoresis, Ellen is our resident expert in the shipping of samples to external laboratories for specialized assays. She participates in quality assurance and reference interval improvements, and was a major force in completing the recently updated laboratory manual.

Originally from Chatham, New Brunswick, Ellen moved to Halifax to complete a Bachelor of Science degree from Dalhousie University and a Registered Technologist diploma from the Nova Scotia Institute of Technology. Ellen is married to Gerard, a firefighter, and they have three children - Beth, Kate and Robbie. Completing the family are Heidi, their 3-legged retriever-x dog, and two cats, Andy and Jack. In addition to her busy family life, Ellen finds time to volunteer on various university committees and is vice-president of the Sherwood-Parkdale Skating Club. She loves to read, garden, camp and walk on PEI's beautiful beaches.

A description of Ellen would not be complete without mentioning the fantastic Halloween costumes she dons each year. These are always a much anticipated fun surprise, as her costumes are always innovative and often reflective of current events or celebrities. Ellen has come dressed as Stompin' Tom as well as Marg Delahunte and the 5th Quinlan quint from *This Hour has 22 Minutes*. An all time favorite was her Hurricane Juan costume from 2003, when her outfit consisted of attached items of debris, such as tree branches and roof shingles. More recently, Ellen's 2008 costume was that of Olympic swimmer, Michael Phelps, complete with fake foam muscles and 8 gold medals!



Reader Feedback: The *Diagnostic Update* group invites comments or suggestions for future topics in the newsletter. Please submit your comments to *Dr. Cora Gilroy* (cgilroy@upe.ca), Diagnostic Services, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.