Diagnostic Update

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Fall, Winter & Spring Hours: Monday to Friday - 8:00 am to 5:00 pm
Summer Hours (June 22-August 31, 2015): Monday to Friday - 8:00 am to 4:30 pm
Saturday - Bacteriology 9:00 am to 12:00 pm & Clinical Pathology 8:00 am to 12:00 pm

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In this Issue:

Finding a Tick on a Dog in the Atlantic Provinces – What Should be Done?

By Barbara Horney, Veterinary Clinical Pathologist

Ticks in Atlantic Canada:

Figure 1: *Ixodes* sp. (engorged female) from a dog. Photograph supplied by Dr. Spencer Greenwood, Department of Biomedical Sciences, Atlantic Veterinary College, UPEI.

Ticks are occasionally found on pets in both rural and urban areas of Nova Scotia, New Brunswick and Prince Edward Island (PEI). They may be *Ixodes scapularis* (blacklegged or deer tick, Figure 1), *Dermacentor variabilis* (American dog tick) or others. Of the PEI ticks submitted to the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory for identification, the most common is the blacklegged tick while others are occasionally seen. Therefore, while it is not as common to pick up a tick in PEI as in Nova Scotia, when a tick is found it is usually an adult blacklegged one. Our historical data shows that adult ticks are typically submitted from pets in this region during two time periods: from April to June and from late August to December. Nymphs of *Ixodes scapularis* are active through the summer and have been found in Nova Scotia and New Brunswick but not PEI to date.

What diseases can deer ticks transmit to pets?

A proportion (10-25%) of blacklegged ticks in Atlantic Canada carry *Borrelia burgdorferi*, the Lyme disease agent. A smaller proportion (0-2%) carry *Anaplasma phagocytophilum*. If transmitted to dogs and horses, these agents can result in illness (Lyme disease or Anaplasmosis). Cats appear to be more resistant to these tick borne agents and illness is uncommon. Lyme disease is transmitted only after a long tick feeding period (24-48 hours). Only a small proportion of dogs (5%) appear to develop clinical illness subsequent to transmission of bacteria from the tick.

Should the tick be submitted for identification?

The AVC Diagnostic Services Laboratory discontinued free tick identification due to financial constraints in 2014, but ticks can still be submitted for identification at a cost*. Any ticks submitted will also be forwarded to the federal microbiology laboratory in Winnipeg for testing for the presence of *B. burgdorferi* and *A. phagocytophilum*. The tick identification has a short turn around time but the infectious agent results can be delayed. **The cost-benefit ratio is not high for tick identification or infectious agent testing**. Even if a tick is carrying infectious agents, it does not mean they have been transmitted to the pet. Also, knowing a tick is negative for infectious agents does not mean that a pet did not have exposure to ticks that may have transmitted a disease agent.

How should animals be monitored?

Clinical illness:

Lyme disease in dogs and horses can result in non-specific signs such as fever, stiffness, lameness and swollen joints. A small proportion of dogs can develop renal disease, including proteinuria and renal failure. Other associated clinical signs in horses include uveitis and neurologic disease.

Anaplasmosis in dogs and horses can be subclinical. In both species, anaplasmosis can present as a vague illness with fever, lethargy, malaise, anorexia, and general muscle pain as well as joint pain and lameness. Distal limb edema is reported in horses. Thrombocytopenia is also reported. The disease can be self limiting, resolving in 1 to 2 weeks even without treatment. In acute disease, bacterial clusters (morulae) may be found in neutrophils on blood smear examination.

Testing for antibodies:

SNAP 4Dx Plus test: Validated only for the dog, this test can detect antibodies to *A. phagocytophilum* and to the C6 antigen of *B. burgdorferi*. It can also detect antibodies in the blood of other species such as horses and cats but the sensitivity and specificity of the test is not known. Dogs with clinical Lyme disease are typically positive for antibodies to *B. burgdorferi*, whereas a negative *A. phagocytophilum* antibody test does not rule out clinical anaplasmosis as dogs can become ill prior to the development of an antibody response. The C6 antigen of *B. burgdorferi* is an antigen present only when bacteria are transmitted naturally from ticks; it is not a vaccine antigen. Therefore, a dog vaccinated for Lyme disease will not have antibodies to the C6 antigen, but those with exposure or clinical Lyme disease should be antibody positive if sufficient time (approximately 4-6 weeks after tick exposure) has elapsed.

Quantitative C6 antibody testing: This is an ancillary test recommended following a positive result on the SNAP 4Dx Plus test. This quantitative value determines the significance of a positive SNAP 4Dx Plus test and allows monitoring of antibody levels subsequent to treatment. Treatment of a clinically healthy dog with a positive C6 antibody test is somewhat controversial, as 95% of dogs exposed to the Lyme disease agent remain clinically healthy.

Multiplex testing for antibodies to the Lyme disease agent: A recent test for a range of antibodies to various *B. burgdorferi* antigens (outer surface protein (OSp) A, C or F) can be used for testing both dogs and horses. It offers evaluation of vaccine response, and a differentiation between acute and chronic Lyme disease infection.

Testing for the presence of the infectious agent:

Polymerase chain reaction (PCR) testing for A. phagocytophilum or *B. burgdorferi*: This PCR test may be helpful to detect *A. phagocytophilum* genetic material in ill patients prior to the development of an antibody response. PCR testing is not generally helpful for Lyme disease detection as bacteria may not be present in the tissue or blood of affected animals.

*Please refer to our current Diagnostic Services Laboratory fee schedule or contact our laboratory (902-566-0863) for costs pertaining to specific tests.

Sources of information on tick borne disease:

North Carolina State University Vector Borne Disease Diagnostic Laboratory:

http://www.cvm.ncsu.edu/vhc/csds/ticklab.html

Cornell Multiplex testing:

https://ahdc.vet.cornell.edu/news/lyme.cfm

Health Canada:

http://www.healthycanadians.gc.ca/diseases-conditions-maladies-affections/disease-maladie/lyme/surveillance-eng.php

Our Role in Antimicrobial Stewardship: the Switch to Routine MIC Testing

By Matthew Saab, Veterinary Bacteriology Technologist and Anne Muckle, Veterinary Clinical Bacteriologist

Antimicrobial resistance (AMR) is a serious global threat to the prevention and treatment of bacterial infections in both humans and animals. The Canadian Veterinary Medical Association's position statement on antimicrobial use "recognizes that the emergence of AMR is a global concern" and "strongly supports responsible use of antimicrobials by the veterinary profession to protect both animal and human health and welfare."[1] The Atlantic Veterinary College Diagnostic Services Bacteriology Laboratory has an important role in antimicrobial stewardship at veterinary practices in Atlantic Canada. We have switched to routine minimum inhibitory concentration (MIC) testing in order to provide expert advice regarding appropriate antimicrobial treatment options for your patients. This also will better monitor trends in bacterial antimicrobial resistance in companion animals and livestock in our region.

Previously, our laboratory routinely performed antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. The major difference you'll note between the two methods is in the reported results. The disk diffusion method provides a qualitative (or categorical) result based on the size of the zone of inhibition, classifying the organism as susceptible, moderately susceptible (intermediate), or resistant. MICs instead provide a quantitative (or numerical) result for each drug tested. These values are always interpreted and still reported with the classifications of susceptible, intermediate or resistant. The MIC value represents the lowest concentration of antimicrobial required to kill or inhibit the growth of that particular organism. It can be compared to the susceptibility range and dosage options of the antibiotic. We believe this provides more useful information for veterinary practitioners to select optimal antimicrobial therapy.

You are probably asking yourself, *"Ok, but what does this mean to me and my practice?"* First, we have slightly increased our fee for a "Routine culture and susceptibility with MIC testing". This price change is to help offset the increased costs of using more sophisticated equipment and methods. Second, the reports from our laboratory will now include the MIC for all drugs tested. This information will allow you to see how susceptible, or how resistant, an organism really is, helping you to make a well informed decision for treatment. Third, the increased information captured from each isolate we encounter in our laboratory allows us to more closely monitor trends in bacterial antimicrobial resistance patterns. By monitoring MIC patterns, emerging resistance will be more readily detected, resulting in earlier dissemination of this important information.

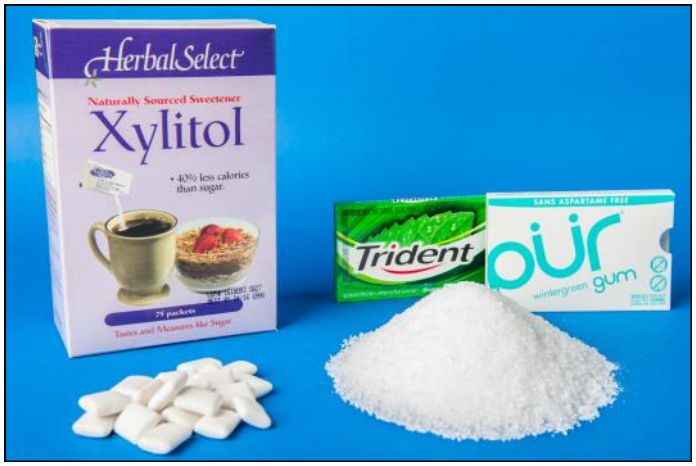
Please contact the Bacteriology Laboratory (902-566-0821) for further information on MIC testing or the interpretation of susceptibility results.

References:

1. Antimicrobial Use in Animals – Position Statement. Canadian Veterinary Medical Association. November 14, 2014. Available at: http://www.canadianveterinarians.net/documents/antimicrobial-use-in-animals. Accessed June 1, 2015.

What's Your Diagnosis?

The sugar substitute shown here is highly toxic to pets. What blood test abnormality does it cause quickly (often within 30 minutes) after ingestion? What is the mechanism?



See page 8 for the answer.

Surveying our Clients - Why? When?

By Jodie Bowmaster, Quality Assurance Manager

The Atlantic Veterinary College (AVC) Diagnostic Services Laboratory is now an ISO/IEC 17025:2005 accredited organization. Accreditation requires that we obtain client feedback on a routine basis to assess the ability of our laboratories to meet each testing need. The survey is designed to assess client satisfaction responses concerning service, testing activities, and the quality management system.

Our client survey was sent to 60 randomly selected clients in December 2014. A discount of 10% was offered to those who completed the survey and attached it with their next sample submission form. A total of 11 surveys were returned. The following tables highlight the responses received.

Diagnostic Services Laboratory Area	Area Used (out of 11 Responses)
Bacteriology	7
Clinical Pathology	10
Histology	7
Parasitology	5
Post Mortem	1
Regional Diagnostic Virology Services (RDVS)	1
Toxicology and Analytical Services (TAS)	2
Veterinary Laboratory Association Quality Assurance Program (VLA-QAP)	0

Survey Question Asked (1 =Strongly Disagree, 5 = Strongly Agree)	Response				
	1	**2**	**3**	**4**	**5**
AVC Diagnostic Services customer service is satisfactory**.**	0	0	0	5	6
Employees are helpful and courteous**.**	0	0	0	4	7
The turnaround time for test results is satisfactory**.**	0	0	0	6	5
Test reports are complete and easy to understand**.**	0	0	2	5	4
I have a high degree of confidence in the test results.	0	0	0	4	7
When I have questions about a case or test results, it is easy to contact the appropriate lab unit/diagnostician for consultation.	0	0	1	5	5
When I have questions about a case or test results, I get a prompt response to my inquiry.	0	0	1	4	6
Courier services are satisfactory.	0	0	4	2	5
The selection of tests provided is satisfactory (to meet/cover the majority of my diagnostic needs.)	0	0	0	7	4
I use other diagnostic laboratories for tests not provided by AVC Diagnostic Services (or in addition to AVC Diagnostic Services).	2	1	0	4	4
The hours of service are satisfactory.	0	0	2	5	4

Other questions asked for additional tests that clients would like to see AVC Diagnostic Services Laboratory offer and asked for any additional comments or suggestions.

If you would like to receive 10% off your next test submission, please contact Liz Dobbin at edobbin@upei.ca or Jodie Bowmaster at jbowmaster@upei.ca for our client survey to complete and attach to your next sample submission form. We will send the survey to another random group of clients with our Christmas cards in December 2015. We thank those who are taking the time to provide us with this valuable feedback to help us serve you better!

Canine Cutaneous Mast Cell Tumors - What is a Passing Grade?

By Andrea Bourque, Veterinary Anatomic Pathologist

In reading recent histopathology reports from our Atlantic Veterinary College (AVC) Diagnostic Services Laboratory, you have probably noticed that we have moved away from the traditional 3 tiered Patnaik grading system to a 2 tiered grading system for canine cutaneous mast cell tumors (MCTs). The aim of the new system was to get rid of those annoying grade 2 (intermediate) MCTs. These have an ambiguous prognosis and left clinicians with little guidance on what to do next, especially when mass resection was incomplete or when tumors were excised with narrow borders.

In veterinary medicine, canine MCTs are one of the few neoplastic conditions with widely accepted tumor grading systems shown to reliably predict behaviour and thus help to guide treatment. Until relatively recently, the Patnaik grading system, proposed in 1984, was the most common system used by pathologists and clinicians. In this system, grade 1 (or well-differentiated) MCTs are considered benign tumors. With complete excision, these tumors are typically associated with long survival times. Depending on the study, survival times of at least several years are generally cited. Grade 3 (or poorly differentiated) MCTs are associated with a poor prognosis. They tend to be locally invasive, have a more rapid growth rate, have a higher risk of metastasis and shorter survival times. On average, survival times of several months are given.

One of the main problems with the Patnaik system is that the majority of cutaneous MCTs have histologic features consistent with an intermediate (or grade 2) degree of differentiation. Several studies have looked at how pathologists grade these tumors and have found significant variation between pathologists in grading the same tumor. These studies also showed that one pathologist may furthermore grade the same tumor differently when examining the same slides at different times - yikes! This is due to the subjectiveness of the Patnaik criteria and the fact that many MCTs have histologic features that overlap, most often between grades 1 and 2. If struggling whether to call a tumor a grade 1 or 2, it is human nature that most pathologists will err on the side of caution and give such tumors a grade of 2. Another frustration with the Patnaik system is that it automatically gives MCTs located in the subcutis a grade of 2, even when tumor cells are well differentiated. The result is that many tumors are classified as grade 2, which is associated in some studies with a 50 % chance of survival at 5 years. Basically, the last sentence translates into "*I don't know what the h%&l this tumor is going to do!*". You might as well flip a coin when trying to prognosticate for a patient diagnosed with a grade 2 mast cell tumor (MCT)!

In an attempt to remedy this situation for pathologists and clinicians alike, a novel 2 tiered grading system was proposed in 2011[1]. It simply divides cutaneous MCTs into 2 categories: low and high grade. A group of 28 board certified pathologists from several American and European surgical biopsy services looked at slides from a large sample group of 95 MCTs from the skin of 95 different dogs. For each dog, surgical treatment was the only treatment and follow-up information was available for at least 4 years after surgery. The new grading system requires assessment of 4 specific, simplified, and largely quantitative criteria. These are mitotic index, the number of multinucleated tumor cells, the number of cells with bizarre or irregularly shaped nuclei, and whether there is marked variation in nuclear size. This is much less subjective than the Patnaik system, resulting in more consistent grading. This report indicated that dogs diagnosed with high grade MCTs had significantly shorter times before additional tumors developed, a higher risk of metastasis, and shorter survival times. The median survival time was less than 4 months for dogs with high grade MCTs and more than 2 years for dogs with low grade MCTs.

Although tumor grading systems can definitely provide useful clinically relevant information, it is important to keep in mind that there are limitations to any system based solely on the microscopic appearance of tumor cells. This is because some tumors just do not follow the rules. A MCT with microscopic features consistent with a low grade may behave in an aggressive manner, and vice versa. Because of these rare exceptions, close follow-up is always warranted. Following diagnoses, additional testing can also be performed on biopsy samples to obtain additional prognostically useful information. MCT prognostic panels are offered at Michigan State University and our AVC Diagnostic Services Laboratory can access this special testing. These panels include immunohistochemical (IHC) testing for c-Kit and ki-67 (a cell proliferation marker), a polymerase chain reaction (PCR) test to detect mutations in c-Kit (a tyrosine kinase receptor), and Agnor special staining (another measure of tumor cell proliferation). MCTs in which c-Kit mutations are detected are associated with a less favorable prognosis. These are generally more aggressive high grade tumors; affected dogs may benefit from treatment with tyrosine kinase inhibiters. Agnor special staining and IHC for ki-67 are thought to be more sensitive indicators of tumor cell proliferation than mitotic index done on routine microscopic examination. They thus may be able to more readily identify high grade tumors when mitotic figures are difficult to

discern. If you are interested in such testing, or have questions regarding these or other tumor grading systems, please do not hesitate to contact us (902-566-0864).

References:

1. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behaviour. *Vet Pathol.* 2011;48:147-155.
2. Gross TL, Ihrke PJ, Walder EJ, Affolter VK. Canine mast cell tumors. In: *Skin Diseases of the Dog and Cat: Clinical and Histopathologic Diagnosis.* 2nd ed. Iowa: Blackwell Science Ltd; 2005:853-858.
3. Miller WH, Griffin CE, Campbell KL. Mast cell tumors. In: *Muller & Kirk's Small Animal Dermatology.* 7th ed. St. Louis: Elsevier Publishing; 2013:806-810.

AVC Diagnostic Services Laboratory at the 2015 Atlantic Provinces Veterinary Conference

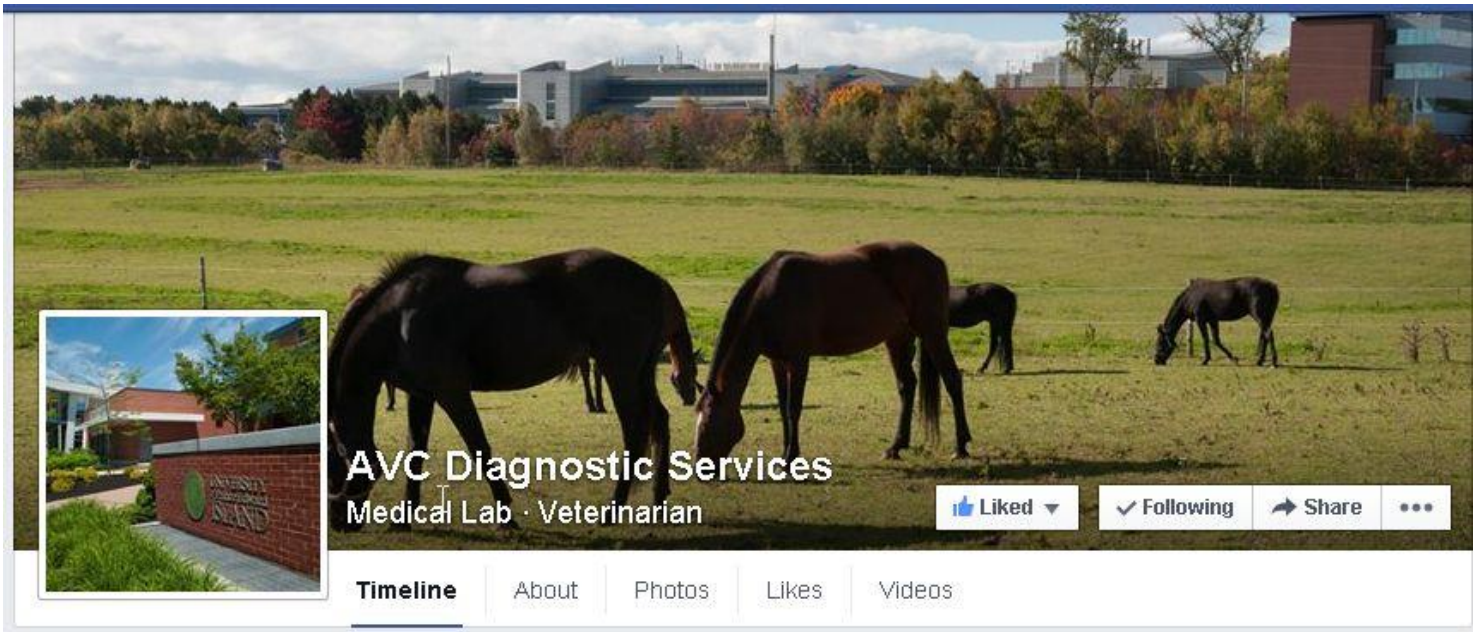
By Cornelia Gilroy, Veterinary Clinical Pathologist

The Atlantic Veterinary College (AVC) Diagnostic Services Laboratory was represented at the annual Atlantic Provinces Veterinary Conference in Halifax, Nova Scotia by Dr. Andrea Bourque, anatomic pathologist and Ms. Liz Dobbin, director. This conference provided a great opportunity to visit with colleagues and friends, as well as receive feedback on our services. Thank you to everyone who invested time to stop by the exhibition booth! We were pleased to offer four draw prizes. Congratulations to our winners:

- First prize of two complimentary surgical biopsies: Dr. Barry MacEachern from Burnside Veterinary Hospital, Dartmouth, Nova Scotia.
- Second prize of two complimentary cytologies: Dr. Robin Bain from Parade Street Animal Hospital Ltd., Yarmouth, Nova Scotia.
- Third prize of one complimentary complete blood count and chemistry profile test: Dr. Deanie Parks from Dr. Deanie Parks Veterinary Office, Blandford, Nova Scotia.
- Fourth prize of an AVC candy dish: Ms. Lisa Purcell, Abegweit Animal Hospital, Charlottetown, Prince Edward Island.

Drs. Shelley Burton and Cora Gilroy, veterinary clinical pathologists and Ms. Ellen McMahon, a veterinary laboratory technologist, provided well-received wet laboratory sessions entitled “Urinalysis - Mining Your Patient's Liquid Gold!” (Figure 1).
We look forward to seeing everyone again next year!

Figure 1: Ms. Ellen McMahon providing a demonstration to participants of the urinalysis wet laboratory session.



The Atlantic Veterinary College Diagnostic Services Laboratory now has a Facebook page. Like us on Facebook!

Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

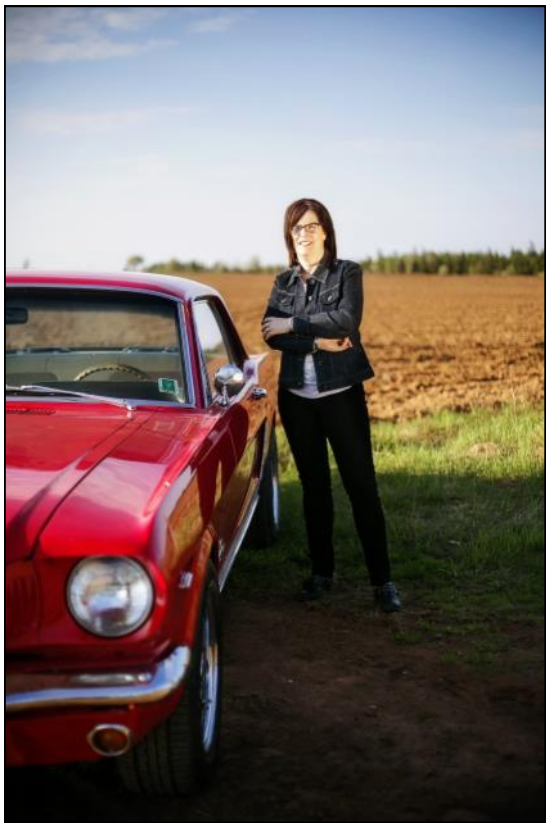
Here are some recent happenings in Diagnostic Services:

- Linda Ruschkowski retired in March 2015 and moved to Saskatchewan after providing many years of service as a hematology technologist. We wish her all the best!
- Several members of Diagnostic Services Laboratory travelled to Halifax in April to participate in the Atlantic Provinces Veterinary Conference (please see full article on page 6).
- The Canadian Animal Health Laboratorians Network annual meeting and World Association of Veterinary Laboratory Diagnosticians meetings were held in Saskatoon, Saskatchewan June 15th - 18th. Representatives from Diagnostic Services Laboratory included Carmencita Yason (clinical virologist) and Liz Dobbin (director).
- Dr. Cora Gilroy, veterinary clinical pathologist, received word that she will be awarded the 2015 Zoetis Carl J. Norden Distinguished Teacher Award, the highest teaching award given by North American veterinary colleges.
- Congratulations to Ulrike Hagmeier, who has been working in our hematology laboratory during the past year, with her new permanent position as the hematology lead technologist.
- Congratulations to Jodie Bowmaster with her transition from acting quality assurance manager to a permanent position in this role.
- Dr. Shannon Martinson, veterinary anatomic pathologist, Dr. Anne Muckle, veterinary clinical bacteriologist and Dr. Cora Gilroy, veterinary clinical pathologist recently all began tenure track positions with the Department of Pathology and Microbiology. We wish them all the best as they begin their new positions!

Staff Focus

Susan O'Connor

By Cornelia Gilroy, Veterinary Clinical Pathologist



Susan O'Connor is well known to many of our clients as she frequently answers the phone for the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory. Originally from Lunenburg, Nova Scotia, Susan obtained her Health Record Science Diploma in 1986 from the Halifax Infirmary. At that time, she began working at the Saint John Regional Hospital in New Brunswick as a Health Record Administrator in the Medical Records Department. She moved to Prince Edward Island in the fall of 1987, where her future husband, John, was living.

Beginning in 1987, Susan worked at the AVC in the Veterinary Teaching Hospital Medical Records as a Health Record Technician and in 1989, moved into a supervisory role of the Admitting/Billing Medical Record Section. Susan joined the Diagnostic Services Laboratory in 1997 as an Administrative Assistant. However, this job title does not encompass all Susan does as she has many roles in our laboratory! In addition to her general administrative duties, Susan's contributions include assisting with sample receiving, data entry into the laboratory management system and sending results to clients from samples sent to referral laboratories. She also handles billing and assisting clients over the phone or in person with questions pertaining to pricing, billing, laboratory results and test requirements. As can be attested by many, Susan contributes in many key areas to help keep our laboratory operating smoothly.

Apart from work, Susan has many interests. Susan and John have two grown children, Taylor and Parker, with whom they have enjoyed many camping and downhill skiing vacations over the years. Susan's current interests include knitting, baking, card making and decorative painting. A great 25th wedding anniversary present to Susan from John was a 1965 red Mustang car which she loves to drive! In future years, Susan and John hope to spend more time travelling both regionally and worldwide, including a Hawaiian cruise and exploring Australia. They hope to join a Recreational Vehicle (RV) missionary group for retired couples to enjoy camping and travel in combination with volunteering time for construction and service work for projects throughout North America. As you can see, Susan likes to be busy and our laboratory is the current lucky recipient of her hard work!

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

Answer to What's Your Diagnosis on page 3: Xylitol causes hypoglycemia (low blood glucose) due to excessive release of insulin from the pancreas. For more information refer to the article "Xylitol toxicity in dogs - an increasing threat in Atlantic Canada" in the February 2015 issue of Diagnostic Update.