Diagnostic Update

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In this Issue:

Xylitol Toxicity in Dogs – an Increasing Threat in Atlantic Canada?

By Shelley Burton, Veterinary Clinical Pathologist

A recent case was seen at the Atlantic Veterinary College of a much loved dog dying after eating homemade cookies containing xylitol. This sad situation illustrated how well meaning owners can tragically presume a safe substance for themselves is fine to give to their pet.

Xylitol is a naturally occurring sugar alcohol commercially extracted from various plants, including hardwood trees.[1] It is found in some brands of sugar free gum and in products such as drugs, candies and toothpaste (Figure 1). It has increasing popularity as it tastes as sweet as regular sugar but has only 2/3 of the calories.[2] Toxic events involving animals is rising and the American Society for the Prevention of Cruelty to Animals (ASPCA) Animal Poison Control Center listed xylitol under people food as one of the top 10 pet toxins in 2013.[3] Xylitol is widely available in Atlantic Canada in sugar free gum as well as in crystalline form at supermarkets and in large bins at bulk food stores.

Figure 1: Chewing gum containing xylitol.

How can this product which is so safe in humans be so toxic to pets? It is mainly because it does not cause pancreatic insulin release in humans but does so strongly in dogs and possibly other animals. Given a sufficient dose (as low as 0.1 mg/kg), severe hypoglycemia in dogs ensues within 30 minutes causing ataxia, seizures and even death. Doses in excess of 0.5 g/kg can cause fatal coagulation abnormalities and hepatotoxicity if the dog initially survives.[2] Laboratory data evaluation shows initial hypoglycemia, sometimes followed by hyperglycemia attributed to a Somogyi phenomenon, a rebound effect mediated by various hormones.[4] Other early changes can include hypokalemia and hypophosphatemia.[4] Once coagulopathy and hepatotoxicity develop, other findings include increased liver enzyme activities and prolonged coagulation test results.[4]

Diagnosis of xylitol toxcosis is presumptive as there is no specific test for it and little time to specifically test for it with such a rapidly developing toxicity. There is no specific antidote and treatment needs to be rapid and aggressive, consisting of dextrose, intravenous fluids, liver protective drugs and sometimes plasma transfusions. The prognosis is good for rapidly treated dogs with only uncomplicated hypoglycemia, but poor for dogs with hepatotoxicosis.

How much xylitol in some brands of sugar free gum is toxic to dogs? This varies greatly between brands. In an average sized 20 kilogram dog, ingestion of as few as 2 pieces of gum could cause severe hypoglycemia while 10 pieces could cause liver failure.[1] When possible, the dose of xylitol ingested should be estimated to guide treatment. A piece of gum can be considered to have 0.3

grams if xylitol is not the first ingredient listed and 190 grams/cup is an estimate for xylitol in baked goods.[2]

What can Atlantic Canadian veterinarians do? Aside from being aware of the signs and treatment of this emerging toxicity, client education is paramount. If pet owners use any products containing xylitol, caution them to store them safely away from pets and avoid sharing human food with them. They should only use veterinary toothpaste to brush their pet's teeth, not human ones. While occasional veterinary products like mouthwashes may contain small amounts of xylitol, they are safe to use in prescribed amounts but could be toxic if ingested in large amounts.[1] Resources include the 24 hour hotlines and websites of the ASPCA Animal Poison Control Centre (1-888-426-4435, www.aspca.org) or the Pet Poison Helpline (1-800-213-6680, www.petpoisonhelpline.com). An excellent online handout for clients is found via the link listed in reference #1 below.

References:

1. Xylitol Toxicity in Dogs. http://www.vcahospitals.com/main/pet-health-information/article/animal-health/xylitol-toxicity-in-dogs/4340
2. Dunayer E. New findings on the effects of xylitol in dogs. *Vet Med.* 2006;December:791-797.
3. Top Pet Toxins of 2013. https://www.aspca.org/pet-care/animal-poison-control/top-pet-toxins-2013
4. Gugler K, Piscitelli CM, Dennis J. Hidden dangers in the kitchen: Common foods toxic to dogs and cats. *Comp Cont Educ Vet*. 2013;35(7):E1-6.

Welcome to the Toxicology and Analytical Services Laboratory

By Jodie Bowmaster, acting Quality Assurance Manager

The Toxicology and Analytical Services (TAS) Laboratory at the Atlantic Veterinary College (AVC) is now officially under the Diagnostic Services Laboratory. It had been part of the AVC Biomedical Sciences Department since 1986. This department had specialized faculty with relevant expertise that was easily drawn upon when needed initially for research done in the laboratory. However, it has more recently functioned primarily as a diagnostic laboratory. The recent application by the TAS laboratory for ISO/IEC 17025:2005 accreditation with the Standards Council of Canada promoted the change in organizational structure.

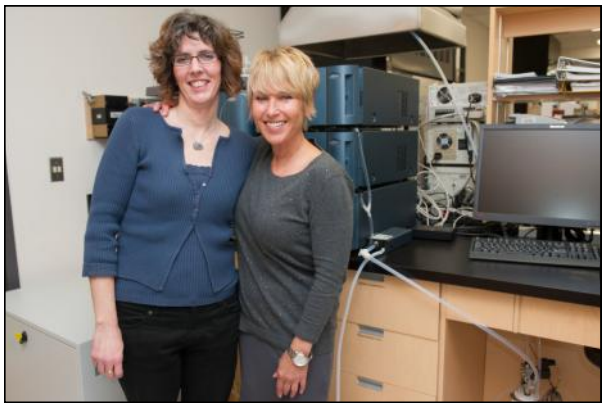


Figure 1: Darlene Mahar and Heather Briand, technologists in the TAS laboratory.

The TAS laboratory operates under a quality management system and has exceptional analytical chemistry testing capabilities. This includes the latest analytical instrumentation such as an Atomic Absorption Spectrophotometer and High Performance Liquid Chromatography instruments. These highly specialized instruments run by skilled technologists allow specialization in testing for residual amounts of pesticides, antibiotics and metals in animal tissue, serum and feeds (Figure 1).

The TAS laboratory also handles unique analytical research projects for many different clients, including large corporations, and expertise for writing and executing analytical method validation protocols, reports and other project requirements.

If you wish to submit samples to the TAS laboratory, the newly revised submission form is found on AVC Diagnostic Services Laboratory website at http://files.upei.ca/avc/QA-F-100-00_MC.pdf. We can also provide this if you contact us by e-mail (TAS@upei.ca) or phone (902) 566-0833. Please complete all necessary fields on the form so we may help you to the best of our ability. We look forward to serving you!

Common routine tests include analysis of:

- Selenium in serum and liver samples
- Vitamin E in serum and liver samples
- Copper in tissue
- Oxytetracycline in salmon tissue and fish feeds
- Emamectin benzoate in salmon tissue and fish feeds
- Lead in blood samples

Antimicrobial Resistance

By Sandra McConkey, Veterinary Clinical Pathologist and Pharmacologist

Antimicrobial resistance is increasing worldwide due to overuse of antimicrobials in both humans and animals. This is concerning as a truly novel family of these drugs has not been discovered in years. Antimicrobial resistance lengthens hospital stays, increases morbidity, affects animal welfare by prolonging suffering and increases the cost of therapy and production.

What is antimicrobial resistance and how does it occur? It is the ability of a microorganism to withstand effects of a normally effective antimicrobial concentration. There are 3 resistance phenotypes in bacteria: 1. Bacteria susceptible to an antimicrobial at the normal dosage. 2. Bacteria with intrinsic resistance containing inherent structural or functional traits that withstand drug actions. 3. Bacteria with acquired resistance. It is this latter resistance phenotype that is of concern to human and animal health.

Acquired resistance occurs by vertical or horizontal transmission of resistance genes. Random mutations in DNA are common during bacterial replication and the mutation rate increases during exposure to antimicrobials. Most mutations are counterproductive and are either corrected or lead to bacterial death. However, occasionally a mutation gives a selective advantage; this is the case with resistance genes acquired during exposure to antimicrobials. Vertically transmitted resistance is typically slow, with multiple mutations needed for clinically significant resistance and succeeding generations of bacteria progressively showing more resistance. Horizontally acquired resistance is more common than vertically acquired resistance. It involves the transfer of plasmids, which are extra-chromosomal self-replicating strands of DNA. Plasmids carrying one or more resistance genes are frequently transferred between bacteria. This constant transfer of genetic material results in random movement of resistant genes between pathogenic, non-pathogenic, environmental and commensal organisms. Horizontally acquired resistance can appear rapidly and may cause a sudden increase in the minimum inhibitory concentration (MIC) of a bacterial population.

There are hundreds of possible resistance genes for every antimicrobial. Resistant genes may alter the antimicrobial target, produce enzymes capable of breaking down the antimicrobial, decrease bacterial uptake or increase bacterial extrusion of the antimicrobial.

Resistance may occur to one or more antimicrobials. Multidrug resistance occurs by cross or co-selection. Cross-selection is a single gene mutation that confers resistance to two or more agents of the same or multiple families of antimicrobials. For example, bacteria that acquire resistance genes during exposure to macrolides may have resistance to all macrolides, or could have resistance to both macrolides and lincosamides. This is because both of these antimicrobial families target ribosomes. Co-selection is multidrug resistance due to the transfer of plasmids carrying multiple resistance genes. Plasmids may contain up to 20-30 resistance genes. These may include a variety of different resistance genes as well as multiple copies of a single resistance gene and/or genes conferring resistance to different antimicrobial families. The variety of resistance genes within a plasmid may or may not be random. For example, pigs treated with tylosin or tetracycline may acquire the resistance genes *ermB* or *tetM. ErmB,* a resistance gene to macrolides, and *tetM,* a resistance gene to tetracycline, are typically located on plasmids that also carry *VanA* which is a resistance gene to vancomycin. Vancomycin is an important antimicrobial drug in human medicine used to treat *C. difficile* infections. Thus, when pigs are treated with macrolides or tetracyclines, there is a chance that their bacteria will develop resistance to vancomycin even though vancomycin had not been used in the pigs.

Why does resistance occur? When an antimicrobial is used to treat a patient, the susceptible organisms are killed or have their growth inhibited, but resistant organisms survive and multiply. We essentially select for resistance by drug usage. Selection for resistance is most common with repeated exposure to sub-optimal concentrations of antimicrobials which are not sufficiently efficacious. This occurs when the antimicrobial is inappropriate for the organism or the infection location. Sub-optimal concentrations also occur if treating the particular strain of bacteria requires a higher drug dosage or dosing frequency. Owner compliance may be poor and there could be missed treatments, altered doses or early discontinuation of therapy. The patient's immune system may also be insufficient. In addition, every treated animal has a variety of drug concentrations throughout the body reflecting penetration into different tissues. While treating a target infectious agent, commensal organisms are exposed to sub-optimal antimicrobial concentrations and they can develop resistance.

What can we do? The most effective way to decrease resistance is to use fewer antimicrobials. Fortunately, when we stop using an antimicrobial, resistance within bacteria decreases. This is because the resistance genes in the plasmids conferring a selective advantage to bacteria during antimicrobial therapy often become a metabolic burden when antimicrobials are not present. International, national and provincial groups are working towards decreasing resistance

through initiatives to encourage decreased use of antimicrobials. In an upcoming Diagnostic Update issue, I'll review prudent use of antimicrobials.

Suggested Reading:

1. Boerlin P, White D. Antimicrobial resistance and its epidemiology. In: Giguere S, Prescott JF, Dowling PM, eds. *Antimicrobial Therapy in Veterinary Medicine.* 5th ed. Ames, IA: John Wiley & Sons Inc.; 2013:21-40.
2. Boothe D. Principles of antimicrobial therapy. In: Boothe D, ed. *Small Animal Clinical Pharmacology and Therapeutics.* 2nd ed. St. Louis. MO: Elsevier Saunders; 2012:128-188.
3. Guardabassi L, Kruse H. Principles of prudent and rational use of antimicrobials in animals. In: Guardabassi L, Jensen LB, Kruse H, eds. *Guide to Antimicrobial Use in Animals*. Ames, IA: Blackwell Publishing; 2008:1-12.
4. World Health Organization. Antimicrobial Resistance: Global report on surveillance, 2014. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1

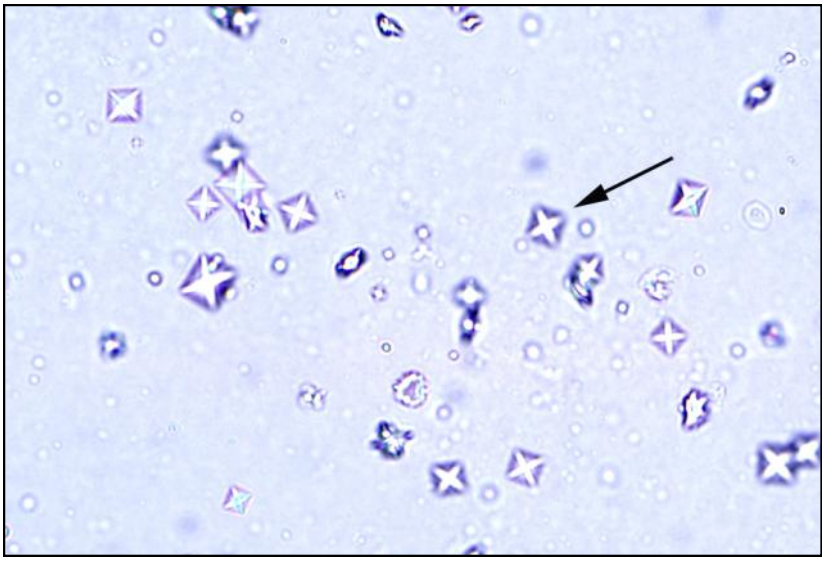
Moving towards Minimum Inhibitory Concentration (MIC) Testing

The Diagnostic Services Bacteriology Laboratory has an important role in antimicrobial stewardship at veterinary practices in Atlantic Canada. Soon we will be switching from our currently used Kirby-Bauer disk diffusion method to routine MIC testing in order to provide quantitative information regarding appropriate antimicrobial treatment options for your patients. We plan to provide more information to you regarding our transition to MICs and our continued role in antimicrobial stewardship in the next few months.

What's Your Diagnosis?

What is the structure indicated by the arrow?

Urine sediment from a dog. Unstained, x 40 objective.



See page 8 for the answer.

Goodbye, Dr. López!

By María Forzán, Wildlife Pathologist

One of our founding faculty, Dr. Alfonso López Mayagoitia, retired this past January after 26 years of working at the Atlantic Veterinary College (AVC) as a professor, researcher and diagnostician. A native of Mexico City, Dr. López graduated as a veterinarian from the Universidad de Taumaulipas, in northern Mexico, in 1973. Following what would become a career long interest in respiratory pathology, Dr. López obtained his MSc and PhD degrees from the University of Guelph working on the effects of multi-pathogen interactions in respiratory disease of cattle. After teaching for several years in his native Mexico, Dr. López returned to Canada in the mid-80s, this time to work for Alberta's Environmental Centre focusing on inhalation toxicology. His time in Mexico had been well spent. He had set up the diagnostic laboratory of the Universidad de Tamaulipas and began the graduate program in anatomic pathology at the Universidad Nacional Autónoma de México in Mexico City. By the time he arrived at the newly formed AVC in 1988, Dr. López had a well established research program in respiratory pathology, an international reputation as a teacher and years of experience as a diagnostician. Always passionate about teaching and a keen technical innovator, he is always one of the first to embrace new technology and modernize his lectures - leading (or occasionally dragging) other professors to do the same and helping the discipline of pathology receive some of the best student evaluations at the AVC. Dr. López has received numerous national and international teaching awards and honorary recognitions. He was the first faculty member in our university to receive a prestigious Canadian International Development Agency grant. His multiple agreements with other universities have made it possible for numerous faculty and veterinary students to train in Canada and abroad. But he is most proud of the chapters he has contributed to several textbooks in Spanish and, most especially, his chapter in Pathologic Basis of Veterinary Disease. The book, originally edited by his mentor and our founding Dean, Dr. R.G. Thomson, has become *the* textbook for veterinary pathology not only in Canada but in many countries, including Dr. López's native Mexico.

Although Dr. López and his wife of 33 years, Rosalie Finney, plan to move to Ontario to be closer to their daughter, Adriana, he will continue lecturing around the world and perhaps even visit us now and again. Good travels, Dr. López, and a heartfelt thank you from all of us you have helped along the way.

ISO Accreditation at the Atlantic Veterinary College

By Jodie Bowmaster, acting Quality Assurance Manager

The Atlantic Veterinary College (AVC) Diagnostic Services Regional Diagnostic Virology Services (RDVS) Laboratory as well as the Toxicology and Analytical Services (TAS) Laboratory have successfully achieved ISO/IEC 17025:2005 accreditation with the Standards Council of Canada (SCC).

What does ISO/IEC 17025:2005 accreditation mean?

ISO/IEC 17025:2005 refers to an internationally accepted standard to demonstrate technical competence in laboratories performing specific tests, calibrations and/or measurements. ISO stands for the International Organization for Standardization headquartered in Switzerland; the ISO term is derived from the Greek word, *isos*, meaning equal. The IEC term stands for the International Electrotechnical Commission. The SCC is one accreditation body in Canada that provides accreditation services to laboratories. It assesses factors relevant to the ability to produce precise accurate test and calibration data; including:

- Technical competence of staff verified through regular proficiency testing or inter-laboratory comparisons
- Validity and use of appropriate test methods
- Traceability of measurements and calibrations to national standards such as National Institute of Standards and Technology
- Suitability, calibration and maintenance of test equipment
- Quality assurance of test and calibration results
- Testing environment, sampling & handling and transportation of test samples

- Communication with customers, necessity to gain feedback and continual improvement.

How does this benefit you as a user of the AVC Diagnostic Services Laboratory?

- Minimizes risk - you are choosing a technically competent laboratory with a sound quality management system in place.
- Avoids expensive re-testing as you will have confidence in our results - your patient's sample has been thoroughly evaluated by an independent, competent testing laboratory that has been assessed by the SCC.
- Recognition by government or other regulatory bodies - results from our laboratory can be used with confidence as accredited laboratories receive regulatory and international recognition.

We look forward to serving you with our new ISO accreditation status!

Leonard Doucette - Happy Retirement!

By Andrea Bourque, Veterinary Anatomic Pathologist

It is truly the end of an era. Leonard Doucette officially retired in August 2014. A little history: In the early 80's, Len was hired at the PEI Provincial Diagnostic Laboratory as a post-mortem technician. When the Atlantic Veterinary College (AVC) was under construction in 1987, Len was hired as the first postmortem technician by our first Dean, Dr. Reg Thomson (also a pathologist). Under Dr. Thomson's careful supervision, Leonard was given the monumental task of setting up a brand new, state of the art, postmortem laboratory. In addition to being a place where veterinary students would be trained, this laboratory would be a functional diagnostic laboratory servicing the Island's agricultural community and the general public, replacing the older provincial laboratory. Both Len and Dr. Thomson succeeded admirably in this task. Even now, almost 30 years later, in large part due to Len's conscientious efforts and care, our laboratory is still technologically current, functional and in many ways, the envy of other institutions.

In his illustrious career at the AVC, Leonard has seen approximately 1400 veterinarians graduate, most of which have spent time on the postmortem floor under his watchful eye. In addition, he has left his mark on countless residents, interns, and graduate students, all who have benefited from his experience and advice. His incredible work ethic, conscientious attitude and genuine enjoyment in his work have made him an indispensible asset. In addition to being able to thoroughly dismantle everything from a draft horse to a budgie in record time, his unique attitude, and job devotion have aided the laboratory to adapt and evolve to the safe, highly functional workplace we currently enjoy.

Yes, it is true - Len could be a little crusty at times. However, working with demanding pathologists and students for almost 30 years would try the patience of an angel! Personally, I have worked with Len on the postmortem floor as a veterinary student, a pathology resident, and in the last 10 years as his direct supervisor. His interest in each case, his keen eye to detail and years of experience made him an asset on the postmortem floor. Even in his last days on the floor with me, he was still trying to help me improve my knife skills - something must finally have taken because I have not spilled my own blood in ages!

Leonard will be missed by all. However, we somewhat jealously wish him well in this well-deserved and exciting time as he spends more time enjoying his non-pathological interests. These include a new interest in travel, photography, metal detecting, looking for native artifacts and old coins, hunting and taxidermy. Okay, the last activities are still a little pathological, but I think we may have left our mark on Len too. Although still often in camouflage, his time spent with treehugger-types in the last many years may have softened him up a little, as he tends to take more pictures than lives now.

Have an excellent retirement fella! And…drop by now and then.

Diagnostic Services at the Atlantic Veterinary College Leading North America in Bacterial Identification

By Jan Giles and Lorraine Lund, Veterinary Laboratory Technologists, Anne Muckle, Veterinary Clinical Bacteriologist and Liz Dobbin, Director of Diagnostic Services

Figure 1: MALDI-TOF mass spectrometer.

In 2008, the Diagnostic Services Laboratory at the Atlantic Veterinary College (AVC) was the first veterinary diagnostic laboratory in North America to use matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) to identify terrestrial bacteria (Figure 1). MALDI-TOF uses mass spectrometry to identify bacteria based on the mass-to-charge ratio of expressed proteins. MALDI-TOF has improved the Bacteriology Laboratory's turn-around time for reporting of all major pathogens, with preliminary reports being sent to clients within 24 hours. In addition, the large MALDI-TOF database of organisms has allowed improved diagnostic capabilities by identifying organisms to the species level that were previously reported as unknown.

Today the bacteriologists, researchers and technologists at the AVC Diagnostic Services Laboratory continue to lead the way in bacterial identification with the expansion of the MALDI-TOF database for aquatic organisms. Normally, the identification of aquatic pathogens is a time consuming process as they take longer to grow than mammalian pathogens and there is a lack of rapid tests on the market for verification which necessitates the use of biochemical testing. The diagnostic team at the AVC have been able to validate the MALDI-TOF process for several major pathogens of cold-water species crucial to the aquaculture industry.

The validation process involves the confirmation of identification by 16s sequencing for each bacterial isolate to be added to the database. Next, a characteristic protein spectrum is generated for each isolate via MALDI-TOF mass spectrometry and this profile is added to the database. This is followed by the statistical test validation of the enhanced database by running both unknowns and previously identified isolates: a first in veterinary aquatic diagnostics. The MALDI-TOF allows for the identification and screening of multiple isolates, critical for both rapid diagnosis and pathogen screening for fish health certification.

The diagnostic team at the AVC has recognized the MALDI-TOF as a valuable tool in the identification of bacteria. However, no instrument can take the place of the judgement of a skilled diagnostic team. The identification of bacteria remains a complex process. Verification of the MALDI-TOF results is sometimes required. Fortunately, the AVC Diagnostic Services Laboratory has a strong diagnostic team to perform this verification when needed.

Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

Here are some recent happenings in Diagnostic Services:

- Drs. Shelley Burton, Cornelia Gilroy, Barbara Horney and Sandra McConkey had fun preparing the 2014 Clinical Pathology Practice Quiz (CPPQ). The CPPQ is a collaborative effort between the University of PEI, the University of Guelph, Michigan State University and Oklahoma State University. It helps clinical pathology trainees preparing to sit the American College of Veterinary Pathologists certifying examination.
- We welcomed three new technologists to the Diagnostic Services Laboratory! Ulrike Hagmeier is working in hematology; Garth Arsenault is in the post-mortem laboratory and Matthew Saab is working in bacteriology.
- We also welcomed Dr. Laura Ross, who began a 1 year Post Graduate Diploma in Anatomic Pathology.
- The Toxicology and Analytical Services (TAS) Laboratory has joined Diagnostic Services (please see full article on page 2).
- The pathologists welcomed medicine and surgical residents from the Atlantic Veterinary College (AVC) in December 2014 for a week of training in pathology needed to meet their residency requirements.
- We wish all the best to Leonard Doucette, post-mortem technologist, and Dr. Alfonso López, veterinary anatomic pathologist, who both retired after many years of service at the AVC (please see full articles for Leonard on page 6 and for Dr. López on page 5).
- The TAS Laboratory and the Regional Diagnostic Virology Services Laboratory of the Diagnostic Services Laboratory has achieved ISO accreditation (please see full article on page 5).

Staff Focus

Pam Maloney

By Cornelia Gilroy, Veterinary Clinical Pathologist

Pam Maloney has been a great asset in several areas of our Diagnostic Services Laboratory. Pam was raised on a dairy farm in Neustadt, Ontario, and obtained her Medical Laboratory Technology training at the former National Defence Medical Centre in Ottawa. After 7 years of service in the military as a Medical Laboratory Technologist, Pam devoted several years to her family which included her husband, Bob, and their two young sons, David and Neil.

Pam and Bob moved to Prince Edward Island in 1988. Bob had accepted a job as a parasitology technologist in the Diagnostic Services Laboratory at the Atlantic Veterinary College (AVC) where he worked until his retirement in 2008. Meanwhile, Pam worked in technical services management at the former Diagnostic Chemicals Limited company for 15 years. Looking for new challenges, she began working as a chemistry technologist at the Queen Elizabeth Hospital in 2003. We were then lucky that she accepted a position in chemistry in the Diagnostic Services Laboratory at the AVC in 2006. Always ready for growth and new experiences, Pam expanded her technical skills by working in our virology laboratory for a year in 2012 and is also currently working there. Regardless of where Pam is working, we can be sure that she brings her skills, professionalism and attention to detail to bear. A superb result is the outcome!

Pam has many interests apart from work. Now that her 2 sons are grown men, Pam has more time to devote to her passion of cycling. In addition to many Maritime cycle tours, Pam and Bob have enjoyed riding from Ottawa to Charlottetown and along the Camino de Santiago in Spain. Another highlight was 6 weeks of cycling approximately 2500 km along the beautiful Rhine and Danube Rivers in Germany. Pam also plays the clarinet in the Second Chances Band of Prince Edward Island. Never wanting to be bored, her future aspirations include taking courses in cycle touring leadership and bicycle mechanics.

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, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.

Answer to What's Your Diagnosis on page 4: Calcium oxalate dihydrate crystal