Diagnostic Update

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In this Issue:

AVC Diagnostic Services at the 2011 Atlantic Provinces Veterinary Conference

By Noel Clancey, Veterinary Clinical Pathologist

An enjoyable time was had by all who participated at the 2011 Atlantic Provinces Veterinary Conference (APVC) in Halifax, Nova Scotia. Dr. Andrea Bourque, Dr. Noel Clancey and Mr. Dennis Olexson represented Diagnostic Services at an exhibition booth during the conference. We would like to thank everyone who invested time to stop by the booth and say hello. This was a great opportunity to receive feedback from clients, as well as catch up with many colleagues and friends. Diagnostic Services was pleased to offer three draw prizes. Warm congratulations to our three winners:

Figure 1: Our Diagnostic Services booth at the APVC this past April. Representing Diagnostic Services was Dr. Andrea Bourque (pictured centre) with AVC Clinical Pathologist Dr. Barb Horney and Dr. Joey Yazer, AVC Class of 2004.

- First Prize of two complimentary biopsies: Sharon Townes from Petworks Veterinary Hospital and Pet Resort, Dartmouth, NS.
- Second Prize of two complimentary cytologies: Lisa Purcell from Abegweit Animal Hospital, Charlottetown, PEI.
- Third Prize of one complimentary complete blood count, chemistry profile and endocrinology test: Aimee Cross from Pinegrove Animal Hospital, Bridgewater, NS.

Drs. Hans Gelens and Sandra McConkey provided two excellent lectures entitled “Hemolytic Anemia: What Now? Current Methods for Diagnosis and Treatment for IMHA” and “Atypical Cells?: The Diagnosis and Treatment of Leukemia in Companion Animals”. Dr. Shelley Burton and Ms. Andrea Chisholm Jack provided a well-received wet-laboratory entitled, “Transfusions - How to Do Them, What to Watch For and How to Set up a Clinic Blood Donor Program”.

We are already looking forward to next year’s conference!

Figure 2: Even Jake, seen here taking a short break from being a model blood donor, was smiling during the APVC! Jake shares his home with Drs. Marti Hopson and Peter Moak, both members of the AVC.

Serum Thyroid-Stimulating Hormone in Cats

By Barbara Horney, Veterinary Clinical Pathologist

Spontaneous hypothyroidism is uncommon in cats[1] unlike the situation in dogs. As a result, there has not been a great demand for the measurement of serum thyroid-stimulating hormone (TSH) in cats. The common diagnosis and treatment of feline hyperthyroidism, and the increasing availability of radioiodine treatment option for this condition has led to a need to evaluate cats for possible iatrogenic hypothyroidism. Similar to dogs, feline serum thyroxine (T4) concentration can be below the reference interval in association with stress, medication administration and non-thyroidal illness as well as with true hypothyroidism, so a low T4 alone is not sufficient to diagnose hypothyroidism. However, a high TSH in addition to a low T4 is supportive of true hypothyroidism.

Peterson[2] reported that a transient subnormal serum T4 concentration is typical following radioiodine therapy. Serum T4 concentration was found to return to within the reference interval in 85% of patients within 2 weeks and 95% within 3 months. Less than 5% of radioiodine treated cats develop true hypothyroidism with clinical signs of lethargy, seborrhea, matted hair and increased weight in 2-4 months. These hypothyroid cats typically have a low T4 and often an increased serum TSH concentration.

The canine TSH assay has been used to measure feline TSH concentrations.[3,4] In our laboratory, the serum TSH reference interval for cats is 0-0.3 ng/ml and cats with TSH greater than 1.0 ng/ml are considered to be hypothyroid. Values between 0.3 and 1.0 ng/ml are considered to be equivocal.

Summary:

1. Transient low serum T4 can occur in cats following radioiodine therapy.
2. The serum T4 will return to within the reference interval in 85% of patients within 2 weeks and 95% within 3 months.
3. If low serum T4 persists in a cat, especially in association with clinical signs suggestive of hypothyroidism, evaluation of serum TSH may aid in the identification of true hypothyroidism.
4. A serum TSH concentration greater than 1.0 ng/ml is supportive of a diagnosis of hypothyroidism. A serum TSH concentration less than 0.3 ng/ml tends to rule out hypothyroidism and a value between 0.3 ng/ml and 1.0 ng/ml is equivocal.

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3. Blois S, et al. Use of thyroid scintigraphy and pituitary immunehistochemistry in the diagnosis of spontaneous hypothyroidism in a mature cat. *J Fel Med Surg*. 2010;12:156-160
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Adverse Drug Reactions

By Sandra McConkey, Veterinary Clinical Pathologist and Pharmacologist

The World Health Organization defines adverse drug reactions (ADR) as "noxious or unintended effects of a drug that occur at an appropriate dose used for prophylaxis, diagnosis, or therapy". Adverse drug reactions are common. Approximately 6.5% of all admissions to human hospitals in the western world are due to an ADR and they are the fourth highest cause of death in the USA. We don't know how many ADR occur in veterinary medicine, but if you practice veterinary medicine and prescribe drugs, then you have likely caused an ADR.

Adverse drug reactions can be classified as 1) dose-dependent or 2) dose-independent (idiosyncratic).

Dose dependent ADR

Most ADR (>75%) are dose dependent. These are predictable; the higher the dose, the more patients affected and the more severe the reaction. This type of ADR is identified during drug trials and is described on the drug label or insert. These reactions are often avoidable but patients may be hypersusceptible due to altered pharmacokinetics from concurrent disease, genetic variability or interaction with other medications.

Dose dependent reactions can be pharmacological or chemical based. Pharmacological based reactions are undesired pharmacologic effects of a drug acting on a specific target or receptor. This includes exaggerated primary responses (such

as a drug that is used to lower blood pressure causing hypotension) or unavoidable secondary effects (such as non steroidal anti-inflammatory drugs, which function by inhibiting prostaglandins but inhibition of some prostaglandins causes gastric ulceration or decreased renal perfusion).

Chemical based dose-dependent ADR occur due to intrinsic-chemical properties of a drug or its metabolites. Some drug metabolites are toxic but of no consequence because they are quickly removed. Toxicity can occur if an individual is deficient in the detoxification system or the process is overwhelmed by an overdose. The clinical signs of these ADR usually reflect where the toxic metabolite is produced (often the liver) and the type of reaction, which may be oxidative damage or nonspecific binding of the metabolite to nearby proteins, nucleic acids or lipids. An example is acetaminophen overdose in dogs which can cause centrolobular hepatonecrosis because the oxidative metabolite NAPQI is produced in centrolobular areas of the liver and will bind to random hepatocellular proteins if the antioxidant glutathione is in insufficient quantities.

Treatment for dose-related ADR includes discontinuing the drug immediately, supportive therapy, treating pharmacological effects in pharmacological dose related reactions or enhancing cellular protective mechanisms (often with antioxidants) in chemical based reactions. Drugs that have caused dose-related reactions can be reintroduced at a lower dose once the reaction has resolved.

Dose-independent reactions

Idiosyncratic reactions are unpredictable and occur in individuals with genetic polymorphisms relating to drug metabolism. They are dose-independent and develop at low doses in susceptible individuals but do not occur at any dose in animals lacking these polymorphisms. Idiosyncratic reactions are often not discovered until a drug has been used by millions of patients. Therefore they may not be initially listed on a drug label or insert. These reactions are typically immune-mediated processes related to drug-modified proteins or autoantigens. They can be a classic anaphylaxis with a rapid onset and history of a previous exposure, or a delayed reaction that occurs 7-14 days after initiation of therapy. For example, sulfonamides can cause immune-mediated fever, lymphadenopathy or hepatopathy in dogs with particular genetic polymorphisms of enzymes that metabolize sulfonamides. Dogs without these polymorphisms may develop dose related reactions to sulphonamides such as keratoconjunctivitis sicca, but will never develop an idiosyncratic reaction to a drug in this family.

Treatment for dose-independent ADR includes discontinuing the drug immediately and supportive therapy ± corticosteroids. The drug should never be used in the individual again as the animal will react, even at low doses.

Diagnosis of an ADR

1. Is the temporal association of the event with drug treatment appropriate for the type of ADR?
2. Has the suspected ADR been reported previously?
3. Are there other possible explanations for the clinical signs?
4. Has the drug been administered previously to the patient and what was the outcome?
5. Do the signs disappear with drug withdrawal and recur with re-exposure? (May not be ethical or safe to re-expose).
6. Is there evidence of a dosing error or elevated plasma concentration (therapeutic drug monitoring)?
7. Are there predisposing factors in the patient such as a concurrent medication, breed or clinical condition?

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Methicillin-Resistant Staphylococci in Atlantic Canada

By Matthew Saab, Veterinary Bacteriology and Public Health Technologist and C. Anne Muckle, Veterinary Clinical Bacteriologist

In the August 2009 issue of Diagnostic Update, we described methicillin-resistant *Staphylococcus aureus* (MRSA) and the risks to animal and human health. At that time, MRSA had not been detected in patients at the AVC Veterinary Teaching Hospital (AVC-VTH) or in horses in Atlantic Canada. The situation is changing, and we would like to update you on MRSA and the other coagulase-positive staphylococci that are significant in veterinary bacteriology.

Historically, the most commonly isolated coagulase-positive *Staphylococcus* species isolated from animals, other than *S.aureus,* was classified as *S.intermedius*. Within the past decade, genomic sequencing has revealed that *S.intermedius* should be re-classified into three separate organisms making up the *S.intermedius* group (SIG). This has also been referred to as the *S.pseudintermedius* complex: *S.intermedius*, *S.pseudintermedius*, and *S.delphini.* Although readily distinguishable from *S.aureus,* differentiation between members of the SIG causes some difficulty for diagnostic bacteriology laboratories. Traditional identification schemes (biochemical reactions or commercial identification tools) are unreliable and provide ambiguous results. To be consistent with the current state of understanding of the classification of the SIG, the Diagnostic Bacteriology Laboratory at the AVC will be reporting isolates as *S. intermedius* group.

Similarly to *S.aureus*, methicillin-resistant strains of these SIG organisms (MRSIG) are becoming more frequently isolated from veterinary patients. As of the fall of 2009, the Diagnostic Bacteriology Laboratory and the AVC Veterinary Public Health Laboratory routinely screen all clinical coagulase-positive staphylococci isolates for methicillin resistance using previously described methods. In 2010, 294 SIG isolates were isolated from a variety of species and sites, with the majority from dogs and their ears. Of these 294 SIG isolates, 3.7% (from 11 animals) were methicillin-resistant. In addition to isolating MRSIG in 2010, methicillin-resistant *S.aureus* was isolated from 3 animals: a horse, a dog and a cat, from PEI, NS, and NB, respectively. From January to June 2011, MRSIG has been isolated from 11 animals – within one year we've already seen an increase in prevalence!

These organisms can be isolated from both clinically ill (infected) and healthy (colonized carrier) animals. Animal patients with an active MRSIG infection pose the greatest risk to other animals that they may be in close contact with. Fortunately, the potential for zoonotic risk of SIG organisms and/or MRSIG is considered to be relatively low, even for people who are immunocompromised. Such individuals should practice frequent hand washing and avoid close contact with the MRSIG-positive animal until the infection is cleared.

As we are seeing more methicillin-resistant staphylococci being isolated from veterinary specimens, especially from companion animals, it is critical to understand the risks of MRSA and MRSIG infections. Of most importance, MRSIG is not MRSA, and the risk of human infection by MRSIG is relatively low. However, as MRSIG can easily be spread from animal to animal, infection control practices should be in place in any veterinary clinic and also in the animal's home environment. Frequent hand washing and routine cleaning and disinfection procedures are basic but essential prevention steps that should be carried out for all cases seen in a veterinary clinic, regardless of presentation.

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Carryover from Purple-Topped Tubes Affecting Potassium & Calcium – Could this be Occurring in your Clinic?"

By Barbara Horney, Veterinary Clinical Pathologist

Whole blood from a 6 year old castrated male beagle was submitted for routine serum chemistry and a CBC in a red-topped (serum, no anticoagulant) and a purple-topped (potassium EDTA) tube respectively. There were no significant findings on physical examination of this dog which was presented for a general health check. The CBC and serum chemistry results were unremarkable except for a moderate decrease in total calcium (Sample A). A second serum sample was obtained and electrolytes and total calcium were rechecked (Sample B). Results are reported in Table 1.

Table 1: Selected serum chemistry results from a healthy canine patient.

Parameter:	**Sodium**	**Potassium**	**Chloride**	**Calcium**
Units:	mmol/L	mmol/L	mmol/L	mmol/L
Sample A:	154	5.8	111	1.81 (L)
Sample B:	152	4.4	112	2.86
Reference Interval:	*144 - 162*	*3.6 - 6.0*	*106 - 126*	*2.24 - 3.04*

The most likely explanation for the apparent hypocalcemia in this healthy mature dog was inadvertent carryover of a small amount of EDTA from the sample collected for CBC into the serum tube of sample A. An EDTA sample was not collected at the same time as Sample B and there was therefore no possibility of carryover. This second sample had a higher calcium concentration and a lower potassium concentration compared to sample A.

Chemical evaluation of EDTA plasma typically results in high potassium (>20 mmol/L) and low calcium (<1.0 mmol/L) concentrations. This is due to the potassium in the anticoagulant and the chelation of calcium by the EDTA. These concentrations of potassium and calcium are incompatible with life and suspecting that the fluid is from a purple-topped tube is not difficult when these results occur. More subtle changes in serum potassium and calcium may result if a small amount of EDTA is mixed with blood by transfer from the EDTA tube to the serum tube. This could occur if the blood is collected in a syringe and the vacutainer tubes are filled through the rubber stoppers, filling the EDTA tube first and then the serum tube with the same needle. The risk of transfer is increased if there is any negative pressure put on the syringe plunger prior to removal from the EDTA stopper as may happen if the blood volume is small and completely filling the EDTA tube would not leave sufficient blood for the serum tube.

An experiment was performed at our laboratory where small volumes of EDTA blood were mixed with whole blood (I) or serum (II) to evaluate this effect (Table 2).

Table 2: Addition of EDTA whole blood to whole blood and serum.

Parameter (mmol/L):	**Sodium**	**Potassium**	**Chloride**	**Calcium**
I) whole blood/EDTA blood				
1.5 ml (100%)/0 ml	149	4.0	112	2.64
1.35 ml (90%)/0.15ml(10%)	151	7.5	112	1.60
II) serum/EDTA blood				
0.25 ml (100%)/0 ml	146	4.7	112	2.71
0.25 ml (95%)/0.0125ml (5%)	147	6.6	112	2.05
0.25 ml (90%)/0.025 ml (10%)	147	7.9	113	1.53
0.25 ml (80%)/0.050 ml (20%)	146	11.1	112	0.43

Small amounts of EDTA blood added to serum or whole blood resulted in increased potassium and decreased calcium concentrations to clinically significant levels without significant alteration of the sodium or chloride concentrations. As a result of this information the following recommendations can be made:

1. When collecting blood samples with a syringe and inserting the needle through the tops of the vacutainer tubes, fill the red-topped serum tube before the EDTA tube to prevent any possible carryover.
2. In cases with unexpected decreased serum calcium and/or increased serum potassium, consider a possible artifact caused by EDTA blood carryover.

Canine Cutaneous Mast Cell Tumours: Re-Visiting Some Old Ideas

By Shannon Martinson, Veterinary Anatomic Pathologist

Mast cell tumours (MCTs) are among the most commonly diagnosed cutaneous tumours in dogs. While diagnosing cutaneous MCTs generally offers little challenge to pathologists, these neoplasms vary widely in clinical behaviour, necessitating further histologic grading. The difficulty encountered in the application of these grading classification systems is well recognized by pathologists, but clinicians may be unaware of this.

With recent advances in technology, including immunohistochemistry and molecular techniques, pathologists are inundated with information regarding new prognostic indicators for canine cutaneous MCTs. Despite this, the most widely used system is the Patnaik morphological grading system, which was developed in 1984. In this system, which relies on subjective histologic criteria, MCTs are assigned a grade of 1, 2, or 3 based on the depth of the mass within the skin and the degree of cellular differentiation. Well-differentiated (grade 1) MCTs are restricted to the dermis and often are behaviourally benign with surgical excision being curative. Poorly differentiated (grade 3) MCTs are locally invasive, extend into the subcutis, metastasize more frequently, and are associated with a poor survival rate. Intermediate (grade 2) MCTs fall in between these two extremes, and thus exhibit less predictable behaviour.

While the biological behaviour of cutaneous mast cell tumours is reported to be well correlated with histological grade using the Patnaik system, a lack of consistency in the application of this system among pathologists is well recognized.[1,2,3] In two related studies, the degree of agreement between 10 pathologists in assigning a grade to 60 MCTs was only 50.3 %[1] and 62.1%.[2] More recently, a study demonstrated agreement among 28 veterinary pathologists to be 75% for the diagnosis of grade 3 MCTs and less than 64% for the diagnosis of grade 1 and 2 MCTs.[3] This lack of agreement does not imply misuse of the system by different pathologists, but instead reflects the subjectivity of grading. When tumors exhibit features that are borderline between grade 1 and 2 MCTs, pathologists will often err on the side of caution and choose a grade of 2. A diagnosis of grade 2 MCT has been shown to have little prognostic value, because there is nearly a 50/50 chance of survival by 5 years in these patients.[3] Such ambiguous information provides little help in making therapeutic decisions with regard to chemotherapy or in deciding if radical tissue resection should be pursued. The results of these studies do not imply that histologic grade is unimportant, but rather suggest a need to improve the current grading system.

Because histologic grade is considered an important prognostic criterion and is often used as a determinant for adjunct therapy, consensus in grading amongst pathologists and the avoidance of lumping tumours into an intermediate category is imperative. It has very recently been proposed that a 2 tier classification system may be more useful for classifying these common tumours.[3] In this system, MCTs were categorized as either low grade or high grade tumours. The proposed grading system demonstrated 96.8% consistency among 6 pathologists and, perhaps more importantly, was shown to be a better predictor of survival than the traditional Patnaik grading system. While this newly proposed system still needs further validation with larger peer-reviewed studies, the initial results are promising and you may see a change in the way MCTs from your patients are graded in the near future!

References:

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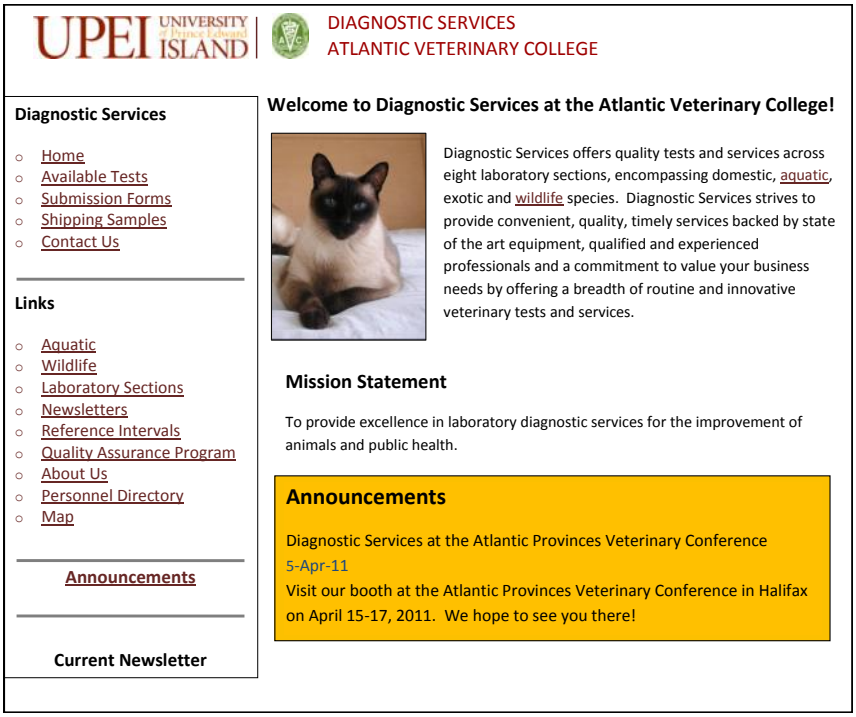
2. Northrup NC *et al*. Variation among pathologists in the histologic grading of canine cutaneous mast cell tumors with uniform use of a single grading reference. *J Vet Diagn Invest*. 2005;17:561–564.
3. Kiupel M *et al*. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol* . 2011;48: 147-155.

Updated Diagnostic Services Website!

By Cora Gilroy, Veterinary Clinical Pathologist

Diagnostic Services website has been revamped to serve our clients in a more effective manner. The goal of the new format is to assist clients find pertinent information. The most requested information is contained in the Diagnostic Services main navigation bar on the left side of the page (Figure 1). A "links" section directly below contains other pertinent content. The current newsletter from Diagnostic Services is available on the home page with archived newsletters found in the "links" section.

The "available tests" section contains an up-to-date searchable file with key information for tests offered by Diagnostic Services, such as turn-around time, days offered, required



sample and handling or storage of the sample. Submission forms in PDF format, as well as guidelines for shipping of samples are some of the other key features. The "laboratory sections" link contains key information pertaining to the various laboratories within Diagnostic Services such as submission guidelines for tests offered by those areas.

We hope the updated website will benefit our clients and we value feedback to help us serve you better! If you have any comments, please direct them to cgilroy@upei.ca.

Figure 1: Home page for Diagnostic Services website.

Laboratory News

By Noel Clancey, Veterinary Clinical Pathologist

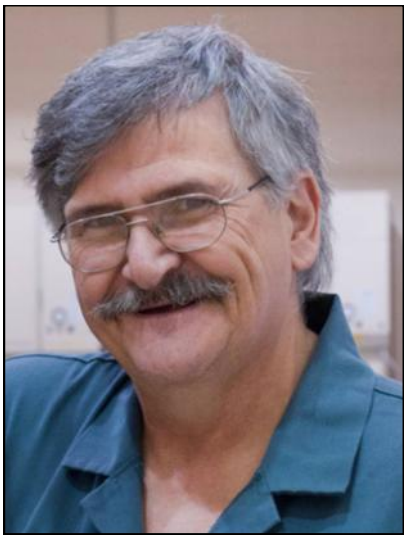
Here are some recent happenings in Diagnostic Services:

- Several members of Diagnostic Services recently traveled to Halifax, Nova Scotia to participate in the Atlantic Provinces Veterinary Conference (please see the full article on page 1).
- Dr. Shelley Burton recently received not one, but two teaching awards. Dr. Burton was awarded the esteemed Hessian Merit Award for Excellence in Teaching. This award requires nomination from three individuals and includes nominees from the entire University of Prince Edward Island faculty. She was also awarded the Vetoquinol Clinical Teaching Award, which goes to a faculty member who promotes interest and enthusiasm in veterinary medicine and who exemplifies teaching excellence in senior clinical rotation; this is voted on by the senior class. Congratulations Dr. Burton!
- The Canadian Animal Health Laboratorians Network (CAHLN) annual meeting was held in Guelph, Ontario during June 5-8, 2011. Several members of Diagnostic Services attended, including bacteriology and mycology technologists Jan Giles and Matt Saab, bacteriologist Dr. Anne Muckle and virologist Dr. Carmencita Yason. The CAHLN was established in 2002 to facilitate exchange of information on animal health diagnostic trends, techniques and research, to provide networking opportunities to identify common issues of concern, and to improve linkages among organizations and scientific staff involved in animal health diagnostic work in Canada.
- Clinical pathology resident Dr. Elizabeth (Betsy) O'Neil is nearing the end of her program and is studiously preparing for the American College of Veterinary Pathologists Board Examination in late September. We all wish her the best of luck. Knock 'em dead Betsy!
- Dr. Barbara Horney will be on sabbatical leave from July to December, 2011. During this time, she will be pursuing research in veterinary medical professionalism and ethics which are special interests of hers. Part of her sabbatical will include study at McGill University in Montreal.

Staff Focus

Leonard Doucette

By Andrea Bourque, Veterinary Anatomic Pathologist



Born in Amherst, NS, and raised in Melrose, NB (just across "the bridge"), most people would be surprised that Leonard Doucette (Len) is actually a "come from away" guy. He moved to North Rustico, PEI, in the early 1970s and has lived in PEI since. In his early days, Len acquired a wide range of work experience which included the commercial fishery and several private abattoirs. Len's unique skill set made him an excellent hire as a postmortem technician in the PEI Provincial Diagnostic Laboratory in the early 80s where he proved to be a valued employee.

When the AVC was under construction in 1987, Len was hired as the first postmortem technician. He was given the monumental task of setting up a brand new, state of the art, postmortem laboratory. In addition to being a place to train veterinary students, it would be a diagnostic laboratory servicing PEI's agricultural community and the general public. Taking this responsibility very seriously, Len, under the direction of the founding Dean (who was also a pathologist - Dr. Reg Thomson), developed our postmortem laboratory. Approximately 25 years later, it is still technologically current and has seen 19 classes of veterinary students pass through its doors.

Since the first days of the AVC, Leonard Doucette has been a cornerstone and valued resource in our postmortem laboratory. 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 patience with students, residents and pathologists are much appreciated. His devotion to his job has allowed the laboratory to evolve and adapt with the changing times to the busy, safe and highly functional workplace we currently enjoy.

In his spare time, Leonard is an avid hunter with interests in taxidermy and wildlife photography. He also may be seen all over the island, on beaches and in farmer's fields, looking for native artifacts and old coins. His studies in the natural history of the Island and its people have taken him from one end of PEI to the other. Although he admits he may be getting a little long in the tooth, we feel that like a good wine, some things only get better with time!

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@upei.ca), Diagnostic Services, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.