

Diagnostic Update



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August 2012

Volume 6, Issue 2



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AVC Diagnostic Services at the 2012 Atlantic Provinces Veterinary Conference

By Cornelia Gilroy, Veterinary Clinical Pathologist

Dr. Andrea Bourque and Mr. Dennis Olexson represented Diagnostic Services at an exhibition booth during the 2012 Atlantic Provinces Veterinary Conference. Thank you to everyone who visited our booth to provide feedback and say hello!

Diagnostic Services was pleased to offer three draw prizes. Congratulations to the three winners:

- First Prize of two complimentary histopathologies: Jessica Sharpe from VetCare Pet Hospital, Riverview, NB.
- Second Prize of two complimentary cytologies: Dr. Barry Falkenham, Seaside Animal Hospital, Lunenburg, NS.
- Third Prize of one complimentary complete blood count and one total body chemistry profile: Megan Dempsey, Bedford Highway Veterinary Hospital, Bedford, NS.



Figure 1: Our Diagnostic Services booth at the APVC. Dr. Andrea Bourque and Mr. Dennis Olexson are visiting with Dr. Mary Ellen Themens from New Brunswick.

Drs. Cornelia Gilroy and Shelley Burton provided a well received morning and afternoon wet laboratory entitled "Blood Smears: Revealing What Lies Within!" This laboratory highlighted the importance of blood smear evaluation using many fascinating cases in addition to having a fun "clicker" quiz on interesting aspects of hematology and a door prize draw.

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



Tentative Diagnosis of *Tritrichomonas foetus* Infection in a Cat

By Gary Conboy, Veterinary Parasitologist, Janet Saunders, Veterinarian, Oriana Raab, Veterinary Medicine Resident
and Spencer Greenwood, Veterinary Parasitologist

A fecal sample was recently submitted from an 8-month-old female cat from Bedford, Nova Scotia. The cat had a history of intermittent diarrhea and had been suspected of having *Giardia* infection. A zinc sulfate centrifugal flotation examination was conducted on the fecal sample and numerous (3+) protozoan trophozoites were detected (Figure 1). The trophozoites were roughly pear-shaped and 11.1-13.9 microns in



Figure 1: Large number of trichomonad trophozoites (arrow) were detected on ZnSO₄ centrifugal flotation of a fecal sample from a cat suffering signs of chronic diarrhea. **Inset:** High magnification of trophozoites.

size. An undulating membrane was visible along the curved edge of the trophozoite (Figure 2). Alive, actively motile trophozoites were recovered on examination of a direct saline fecal smear of the sample. The characteristic rippling wave-like motion of the undulating membrane was visible in the moving trophozoites, indicating that it was a trichomonad and not *Giardia*. The trophozoites were tentatively identified as *Tritrichomonas foetus* based on host, clinical signs and morphology. A sample was collected for culture and confirmation of the identification through PCR to rule out the possibility that the trophozoites were *Pentatrichomonas hominis*, a non-pathogenic trichomonad that closely resembles *T. foetus* in size and morphology.

This case, if confirmed by PCR, represents the first diagnosis of this parasite in a fecal sample in a cat in the Clinical Parasitology Laboratory of Diagnostic Services at the Atlantic Veterinary College Teaching Hospital. In a survey of infection in Humane Society cats, there has been one confirmed *T. foetus* infection to date (Greenwood & Raab, unpublished). *Tritrichomonas foetus* has long been associated with venereal transmission and abortion in cattle. The recognition of *T. foetus* infection as an important cause of large bowel diarrhea in cats is relatively recent (since 1996). Infection appears to be most common in densely housed young cats (< 1 yr of age) of pedigree breeds (especially Siamese and Bengals). Diagnosis of *T. foetus* infection in cats is usually by detection of trophozoites on direct saline fecal smears or by culture of feces. This case was unusual in that trophozoites were seen but are seldom detected by fecal flotation due to the severe damage they suffer from exposure to the high specific gravity flotation media. Equally atypical was the recovery of living trophozoites from a refrigerated sample over 12 hours old. This case illustrates that infection with *T. foetus* should be considered in cases of large bowel diarrhea, especially in young pedigree breed cats originating from large catteries.

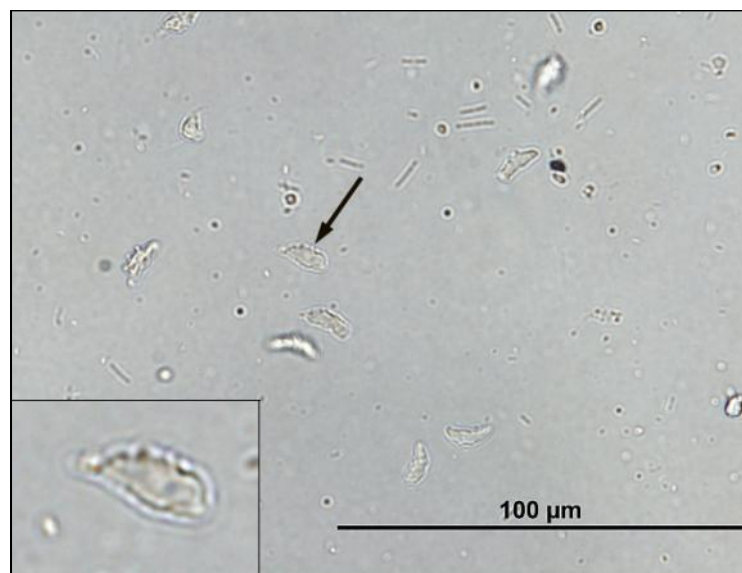


Figure 2: The undulating membrane is the defining morphological feature of a trichomonad trophozoite. However, it is difficult to visualize in non-motile trophozoites recovered on flotation. The undulating membrane appears as an irregular edge (arrow) on the curved surface of the above trophozoite. **Inset:** High magnification of trophozoite.

What are Patented, Generic, Compounded and Pirated Drugs?

By Sandra McConkey, Veterinary Clinical Pathologist and Pharmacologist

Times are changing for drug purchasing. Veterinarians writing a prescription assume the primary responsibility for the medical treatment of that patient; however, the client is not obligated to purchase the drug from the prescribing veterinarian. The client may go to another clinic, a human pharmacy or may purchase the drug through the internet from another country. I had a request recently from a veterinarian wishing to measure serum omeprazole because a client had bought omeprazole online from India and it didn't appear to be working! This scenario is likely to become more common in the days ahead. Veterinarians need to be able to explain the difference between patented, generic, compounded and pirated drugs to their clients.

In Canada, a company owning the patent for a drug has the exclusive right to sell that product for 20 years. Before it can market and sell the drug, the company must first prove its safety and efficacy to the Veterinary Drug Directorate (VDD) of Canada. This is costly; some human drugs cost > \$800 million to bring to market. Drugs passed by the VDD are given a Drug Identification Number (DIN), indicating the right to mass produce and market the drug in Canada. The drug company, packagers, labelers, distributors and all other individuals involved in producing a patented drug must abide by Good Manufacturing Practices (GMP) and comply with all applicable regulations. The company takes responsibility for the safety, stability and efficacy of the product.

Companies producing a generic drug must prove to the VDD that it is bioequivalent to the original patented product to be given a DIN by the VDD. Companies are not required to do safety and efficacy studies for generic drugs. This saves time and millions of dollars. Generic drugs cannot be sold until the patent on the original drug has expired. Like patented drugs, generic drugs are produced in accordance with GMP and other regulations. Producers of generic drugs also take responsibility for the safety, stability and efficacy of their products. The main difference between a patented and generic drug is cost, but the pharmacokinetics of some generic drugs may also differ from the patented product in some animals.

Compounded drugs are NOT generic drugs. Compounding is the mixing of two or more ingredients (of which at least one is a drug or a pharmacologically active component) to create a final product in a form appropriate for dosing. They are mixed for individual patients. We use compounding to produce drugs that are otherwise unavailable (for example potassium bromide or cisapride) or to modify drugs to accommodate a specific patient. Compounded drugs are preferably

made from modifying veterinary drugs, or if that is not possible, human drugs. Only when neither of these is possible can companies use a bulk active pharmaceutical ingredient (API). Compounded drugs are not produced under the laws of GMP. Instead, they are produced in accordance with Health Canada Policy Guidelines regarding compounding. These ensure that patients receive safe and efficacious products, avoid violative food residues and confirm that compounding isn't done to circumvent the regular process of drug application, certification and production. Veterinarians take responsibility for the safety and efficacy of compounded products. The CVMA guidelines have a full description of compounding³.

Pirated drugs are mass produced from bulk APIs in contravention of patent and compounding laws. An API is any substance or mixture of substances that become the active ingredient of a drug. Pirated drugs have not been assessed by Health Canada for bioequivalence, safety or efficacy, and are not produced in accordance with GMP or other regulations. They have no DIN and may be sold under the guise of compounded drugs. The APIs may be imported from a third world country. The World Health Organization has estimated that up to 25% of third world bulk APIs may be substandard or fake drugs. Dispensing pirated drugs is considered to be providing substandard care because there is no proven safety or efficacy.

How veterinary clients purchase drugs is rapidly changing. They are no longer solely reliant on their veterinary clinic to provide a safe and efficacious product. However, veterinarians can still play a role in helping their clients navigate the bewildering choices available and to avoid products with suspect efficacy or safety.

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Hypoglycemia - Artifactual or True?

By Cornelia Gilroy, Veterinary Clinical Pathologist

Serum (red stopper tube) or heparinized plasma (green stopper tube) samples are used by most chemistry analyzers for determining glucose concentration. If the blood cells are not removed from the serum or plasma, they will continue to utilize glucose; the concentration will therefore decrease and will not reflect the true glucose concentration in the patient. The glucose concentration will typically decrease by ~5-10% per hour in a whole blood sample, but this process can be hastened if there are higher than normal numbers of red or white blood cells in the sample. If a sample is not able to be centrifuged within 1 hour, keeping the sample cool can help slow the utilization of glucose by the cells.

The best method to prevent an artifactual hypoglycemia is to centrifuge the sample and separate the serum or heparinized plasma from the cells within 1 hour of sample collection. A less common method is the use of a special blood collection

tube containing sodium fluoride and potassium oxalate (grey stopper tube). The sodium fluoride prevents glycolysis while the oxalate is an anti-coagulant, resulting in a plasma sample that is used for glucose measurement. However, even when these grey topped tubes are used, the plasma glucose concentration can still decrease by ~5-10% in the first hour, after which it appears to remain stable. Samples from the grey stopper tubes cannot be used for assays that use glucose oxidase as the fluoride will inhibit its activity, making the results unreliable.

Please feel free to contact us if you have any questions concerning sample handling prior to submitting a sample!

Reference:

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Canine Histiocytic Disease: The Reader's Digest Version

By Andrea Bourque, Veterinary Anatomic Pathologist

How many times have you received biopsy results with the vague term "histiocytic" disease in the list of differential diagnoses? Have you scratched your head and said, "What am I supposed to do with this?" Maybe this very brief synopsis of canine histiocytic diseases will help to clear the waters.

First, what are histiocytes? They are bone marrow-derived phagocytic cells, and include macrophages and interstitial dendritic cells. The most commonly encountered and clinically important canine histiocytic diseases represent aberrant proliferations of dendritic cells. These cells normally reside in soft tissues throughout the body but are especially prevalent in the skin, lymph nodes and spleen. At these sites, they process and present antigens to sensitized T lymphocytes and thus are important in maintaining normal immunity.

Canine cutaneous histiocytomas (Figure 1) are very common, benign skin lesions which occur as solitary, raised, round, pink to white, partially alopecic and sometimes ulcerated masses. These masses most frequently occur in dogs less than 4 years old and

often spontaneously regress. Very rarely, dogs may develop lesions that clinically and microscopically resemble multiple histiocytomas; these lesions are often persistent and recurrent. Affected dogs have a more guarded prognosis as such cases tend to behave more like reactive histiocytosis and may represent an atypical form of the disease.

Canine reactive histiocytosis is a relatively uncommon idiopathic condition thought to represent a form of immune dysfunction rather than neoplasia. This condition occurs in cutaneous or systemic forms, both of which involve the skin. The lesions of cutaneous reactive histiocytosis are typically restricted to the skin and subcutis. They consist of multiple, non-painful, non-pruritic nodules and plaques which may

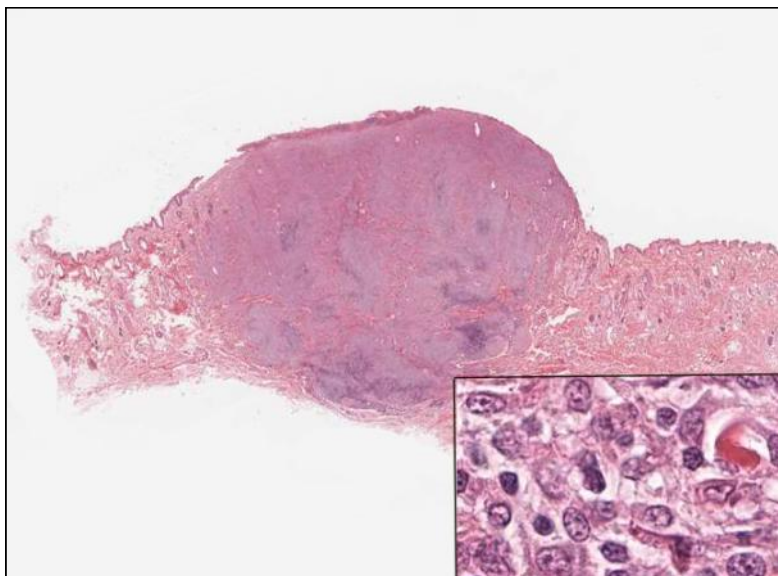


Figure 1: Low magnification image of a cutaneous histiocytoma from a dog. H&E. **Inset:** Histiocytoma. H&E, x 40 objective.

eventually become ulcerated. Lesions may occur anywhere on the body but are most frequent and numerous on the head, neck, scrotum, perineum and extremities. Very rarely, regional lymph nodes may be involved. Less frequently, lesions are restricted to the nasal planum and the mucosa lining the nares, which becomes thickened and reddened. Lesions tend to wax and wane; some may spontaneously regress while new ones develop. Although remissions may occur, most cases are slowly progressive. All breeds are susceptible. Corticosteroids have been useful in some cases to promote resolution of lesions (one reference indicates that approximately 10% of patients will respond¹). Other immunosuppressive therapies have also been used successfully. Long term treatment is generally required.

In addition to skin lesions, dogs with systemic reactive histiocytosis exhibit involvement of other sites, which may include lymph nodes, eyelids, sclera, nasal mucosa, lungs, liver, spleen and bone marrow. Affected dogs may have nodular or diffuse swelling of the nasal mucosa often resulting in respiratory stertor. Ocular lesions are common and resemble bilateral conjunctivitis and episcleritis. Lymphadenopathy is common. Bernese Mountain dogs, Rottweilers, Irish Wolfhounds, Golden and Labrador retrievers are most commonly affected, but all breeds are susceptible. Well-defined age predilections have not been reported. The prognosis for these dogs is generally poor. This disease is progressive and immunosuppressive therapies have been used with limited success.

Histiocytic sarcoma (HS) refers to a relatively common, malignant, locally invasive neoplasm most commonly occurring in Bernese Mountain dogs, Rottweilers, Golden, Labrador and Flat-coated Retrievers. These tumors may initially be localized and often occur in skin and periarticular soft tissues and if excised early, these tend to have the best prognosis. Longer standing tumors have a greater risk of metastasis to regional lymph nodes and visceral sites. Histiocytic sarcomas may also arise within visceral sites (such as the spleen, lung and liver) where the tumor may occur as isolated masses or as diffuse organ enlargement. Central nervous system involvement has also been reported and the Pembroke Welsh Corgi appears to be predisposed to this form. Clinical signs are generally non-specific and depend on the organ/site involved. When metastasis occurs, it is often widespread by the time visceral tumors are identified. There is debate as to whether widely disseminated HS (previously referred to as malignant histiocytosis) represents widespread metastasis or multicentric tumor development. Regardless, these dogs have a very poor prognosis. This disease tends to be rapidly progressive resulting in death or euthanasia; useful chemotherapeutic treatments have not been found. Hemophagocytic HS (Figure 2) is a rare, rapidly progressive, presentation of this disease characterized by fever, weight loss and depression. Lymphadenopathy and hepatosplenomegaly due to neoplastic infiltrates are common. Severe anemia, which is initially regenerative, is characteristic of the disease and is due to marked phagocytosis of erythrocytes by tumor cells. Hypcholesterolemia and hypoalbuminemia are also often reported in affected dogs. This condition usually progresses to pancytopenia as bone marrow infiltration and eventual marrow failure occurs.

Given the widely different prognoses and treatment options, accurately differentiating these entities from one another and from other neoplastic processes is very important. Tissue biopsy of suspect lesions is necessary to make an accurate diagnosis and

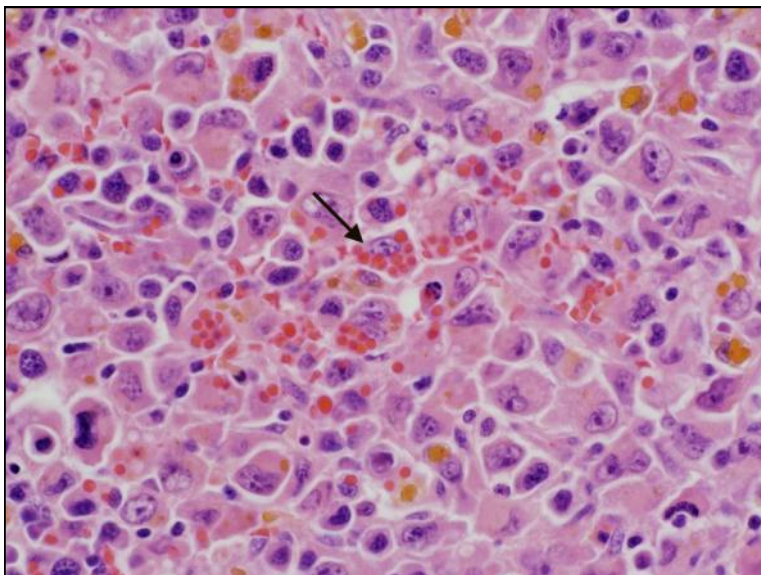


Figure 2: Hemophagocytic histiocytic sarcoma with erythrophagocytosis (arrow) in the spleen from a dog. H&E, x 40 objective.

routine histology is the most common diagnostic tool used. However, making a definitive diagnosis can be, in many cases, challenging. These histiocytic conditions can possess histologic features that resemble reactive or granulomatous inflammatory lesions, lymphoproliferative disease and other round cell sarcomas. Additional testing, such as immunohistochemistry and/or immunocytochemistry, is often required. Histopathology results must always be interpreted in the context of the entire clinical picture to optimize the accuracy of the diagnosis. As with all submissions, a thorough clinical history including a description of the lesion(s), lesion distribution and signalment, is crucial. For example, submission of one skin mass with the appropriate microscopic features from a 4 year dog with a history of "skin lump from a dog", will most likely elicit a diagnosis of histiocytoma from the pathologist. However, if it were known that there are multiple skin nodules present, additional testing would likely be advised as the differential diagnoses would include reactive histiocytosis and cutaneous lymphoma, which have much poorer prognoses and different treatment options.

In closing, diagnosing canine histiocytic disease can be challenging for both clinicians and pathologists. Making an accurate diagnosis is best achieved by collecting good quality tissue biopsies, providing thorough histories with submissions and evaluating your results in the context of your patient. If the results you receive do not make sense with what you see clinically, or the clinical picture changes, you should not hesitate to call and discuss the case with a pathologist. Your input could change how the microscopic lesions are interpreted, and thus influence the disease conditions considered. Remember, the pathologist is looking at a small, close-up snapshot of the problem, while you are looking at the whole 3D movie!

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New Analyzer for Diagnostic Services: What it Means for You!

By Noel Clancey, Veterinary Clinical Pathologist

Diagnostic Services is excited to announce the recent acquisition of the Immulite® 2000 analyzer (Figure 1). The Immulite® system is used to measure most of the hormones requested by our clients, such as cortisol, progesterone and thyroxine (T₄). The new analyzer has a fast throughput as it is able to run up to 200 tests/hour. It also has an automatic clot detection system and automatic re-analysis of samples that fall out of range, including onboard dilution for extremely high values. New to the Immulite® 2000 is the ability to utilize bar-coded sample tubes of two different sizes. This makes the analyzer more flexible to deal with samples such as those from avian or small mammals. A smaller sample volume is another benefit of the new analyzer. Real-time instrument monitoring via the internet allows for fewer unplanned service issues and the analyzer boasts bi-directional interface allowing smooth communication and data downloading to Diagnostic Services' established quality control management system. Collectively, these ensure our clients are receiving prompt, reliably consistent results.



Figure 1: New Immulite® 2000 analyzer acquired by Diagnostic Services.

Perhaps best of all, Diagnostic Services is now able to provide a new Canine Total T₄ assay on the new analyzer. This assay allows for a lower limit of detection of T₄ as well as a much improved analytic sensitivity relative to the previous T₄ assay. Clients have likely observed this lower detection limit on reports (<6.4 nmol/L compared to the previously reported <12.8 nmol/L).

Looking forward, the Immulite® 2000 is able to perform a wide array of assays. Some of these assays include free T₄, adrenocorticotrophic hormone, canine trypsin-like immunoreactivity, folate, cobalamin and various drugs requiring therapeutic monitoring. As we become more familiarized with the new instrument, additional tests are anticipated to be offered in the future.

Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

Here are some recent happenings in Diagnostic Services:

- Congratulations to Kensington Veterinary Clinic, the winners of three free single cytologies for participating in our recent client survey.
- Anne Dover retired in February 2012 after providing many years of service to Diagnostic Services as a hematology technologist. We wish her all the best!
- We want to welcome our new technologists - Jennifer Boutilier in hematology and Maria Vasquez in bacteriology.
- Congratulations to Dr. Shelley Burton, Veterinary Clinical Pathologist and Dr. Gary Conboy, Veterinary Parasitologist, on their promotions from Associate to Full Professor.
- The Canadian Animal Health Laboratorians Network (CAHLN) annual meeting was held in Winnipeg, Manitoba during June 3-6, 2012. Dr. Carmencita Yason represented Diagnostic Services at this meeting. 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.
- Dr. Anne Muckle, Clinical Bacteriologist, attended the Global Development Symposium – Critical Links between Human and Animal Health, May 6-9, 2012, at the Ontario Veterinary College, University of Guelph. The conference focused on interdisciplinary approaches to improving public health and food security worldwide while empowering communities for lasting change.
- Dr. Alfonso Lopez, Veterinary Anatomic Pathologist, was awarded the 2011 Janet Pottie Murray Award for Educational Leadership in recognition of an outstanding leader in education at the University of PEI and for demonstrating a consistent commitment to university teaching.
- Dr. Paul Hanna, Veterinary Anatomic Pathologist, was awarded the 2012 Pfizer J. Norden Distinguished Teacher Award, the highest teaching award given by North American veterinary colleges.
- Dennis Olexson, Diagnostic Services laboratory manager, and Ramona Taylor, histopathology technologist, were both recognized for their commitment and valued dedication for 25 years of service to the University.
- Dr. Barbara Horney returned from sabbatical leave and during that time authored a special report recently published in the Canadian Veterinary Journal (May 2012, 53: 499 - 501) entitled "A call for a national guidance document for veterinary professional conduct in Canada".
- Diagnostic Services has acquired a new analyzer, the Immulite®2000 Immunoassay System that will be used for endocrinology and reproductive testing. Dr. Noel Clancey is overseeing the transition and correlation study that is necessary for this new analyzer to be fully utilized for patients (please see the full article on page 6).
- Congratulations to Dr. Dania Villarnovo, clinical pathology resident, on receiving two awards at the 2012 Atlantic Veterinary College Graduate Studies and Research Days for her presentation entitled "Independent evaluation of a commercially available major cross-matching kit (RapidVet®-H) for use in dogs, cats and horses". Dr. Villarnovo received the Dr. Basil Ikede Award in Diagnostic Veterinary Sciences, as well as a bronze prize in the category of her research (Theme One, Animal Health Research).
- The clinical pathologists welcomed medicine and surgical residents from the AVC and an oncology resident from the Western College of Veterinary Medicine in June 2012 for a week of training in clinical pathology.

Staff Focus

Lorraine Lund

By Shelley Burton, Veterinary Clinical Pathologist and Anne Muckle, Veterinary Bacteriologist



With her cheerful nature and her quick efficiency, Ms. Lorraine Lund is well known in the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory. Lorraine hails from North River, PEI, and has spent her career on the Island. Following a year at the University of PEI, she embarked on a training program in medical laboratory technology at the PEI Provincial Laboratories, graduating in 1971. A 7 year period of work in hematology and bacteriology at this laboratory followed before Lorraine took a 10 year break to raise her 3 children, Kelly, Greg and Bethany. The AVC was fortunate to then have her start her position in the brand new Bacteriology Laboratory on July 2nd, 1987. This means that Lorraine will celebrate 25 years of service this year!

At the AVC, Lorraine's duties involve benchtop identification of organisms and sensitivity testing. She can be found working every Saturday, expertly plating cultures and managing other tasks. Lorraine helps with teaching the 4th year veterinary students on rotations and particularly enjoys visits from clinicians who offer more information on patients. Lorraine loves her work and honestly reports that, "I wake up every morning looking forward to the day!"

Lorraine has many interests outside of work. She has a strong interest in genealogy and has diligently tracked the records of 6 related branches of her family. In fact, she likens her love of genealogy to her love of bacteriology, as the tiny bacteria she works with also have family (phylogenetic) trees! Lorraine loves gardening, traveling and reading historical fiction as well as spending time with her 2 grandchildren. She enjoys a fine glass of wine, especially red wine made by her husband, Chuck.

Although Lorraine is widely respected by colleagues, she does have one flaw – her habit of scooping up pens, scissors and other items throughout the day, resulting in a heavier and heavier lab coat for her and co-workers wondering, "Now...where did I leave my pen?" However, Lorraine's delightful personality, hard work and bacteriology skills more than make up for these kleptomaniac tendencies; we hope to be fortunate to work with her for many more years!

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.