Diagnostic Update

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

Fall, Winter & Spring Hours: Monday to Friday - 8:00 am to 5:00 pm
Summer Hours (June 27-August 31, 2016): 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 4:00 pm

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In this Issue:

Cryptococcus gattii in the Maritimes: The fungus is among us and we want your samples!

By Andrea Bourque, Veterinary Anatomic Pathologist

In July 2014, Department of Natural Resources officers were called by a concerned citizen in Greenwood, Nova Scotia, because a young female white-tailed deer was acting strangely in a residential area. The deer walked in tight circles with a prominent head tilt, was ataxic with a high stepping gait and was not fearful of people. She appeared to be blind, was drooling and dyspneic. The officer euthanized the deer and tissues were sent to the Atlantic Veterinary College (AVC). Dr. Scott McBurney, a pathologist with the Canadian Wildlife Health Cooperative (CWHC), examined the tissues as part of their scanning wildlife health surveillance program. Tissue examination revealed areas of granulomatous pneumonia, lymphadenitis, rhinitis and encephalitis. Each of these inflammatory lesions had myriads of round yeasts with thick mucoid capsules. The microscopic features of these yeasts were consistent with *Cryptococcus* sp. (Figure 1).

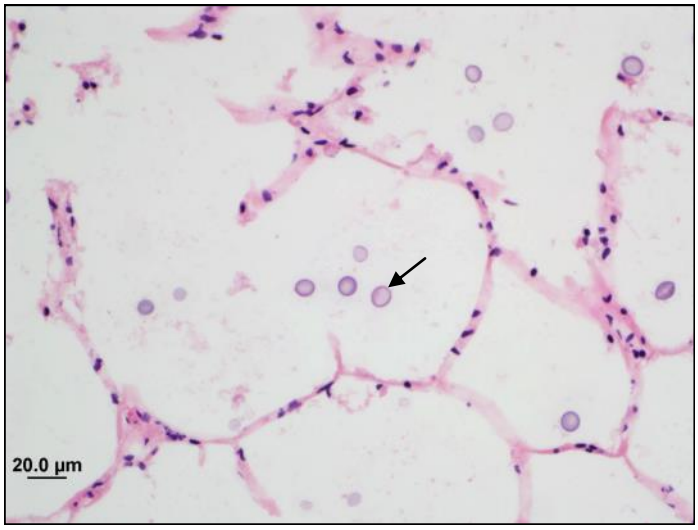

Figure 1: Lung: Widely spaced small ovoid yeasts (arrow) within alveolar spaces (image courtesy of Dr. Nicole Kaiser). H&E.

Because *Cryptococcus* sp. had not been previously reported in deer and because the AVC Diagnostic Services Laboratory has a talented mycologist, Dr. David Overy, further investigation was performed. Following culture of the organism, multilocus genetic sequencing identified it as being a unique variant of *C. gattii*, most similar to genotype VGIIB. To our knowledge, this is the first laboratory confirmed case of *C. gattii* infection in the Atlantic provinces, and the first confirmed case in a deer.[1]

Cryptococcosis in humans and susceptible animals is generally restricted to several species grouped as *C. neoformans-C. gattii* complex; this includes *C. neoformans* var. *neoformans*, *C. neoformans* var. *grubii* and *C. gattii* .[2,3] *Cryptococcus gattii* is further subclassified via genotyping into 4 groups; VGI, VGII, VGIII and VGIV. All have a similar microscopic appearance; genetic sequencing of the organism is required for differentiation.

Until the 1990s, *C. gattii* was considered to be restricted to several tropical and subtropical regions, including Australia, South America, southeast Asia, parts of Africa and an area of California. Since 1999, human hospitals on Vancouver Island have reported a marked increase in laboratory confirmed cases of *C. gattii* infection and the Pacific Northwest is now considered an endemic area.[3] In this same time period, laboratory confirmed cases of *C. gattii* infections in dogs and cats from the area were also common.[4] In general, veterinary cases were diagnosed 2-3 times more frequently than human cases.[4]

Cryptococcus gattii is now considered an emerging pathogen in a broad range of animals. Infection has been reported in domestic cats, dogs, horses, sheep, cattle, koalas, dolphins, gray squirrels, ferrets, birds and marsupials.[3] The organism lives in the environment and can be isolated from the soil, tree bark and decaying organic material. In endemic areas, the fungus has also been isolated from the air, fresh and sea water[2] and has been recovered from car wheel wells and on footwear from high traffic areas. Disruptive environmental activities (like logging and construction), traffic from endemic areas, and movement of bark mulch have all been implicated in the gradual spread of this organism to new areas.[4]

Infection is typically via inhalation of small basidiospores or desiccated yeast cells. Direct infections through skin wounds have rarely been reported, most frequently in cats. A variety of virulence factors have been identified, including the large gelatinous capsule, which gradually enlarges in chronic infections and serves to prevent phagocytosis by macrophages and neutrophils. It is interesting that although *C. neoformans* and *C. gattii* share many virulence factors, *C. gattii* most commonly causes infection in immunocompetent people and animals, while *C. neoformans* primarily infects immune-compromised individuals.[2] *C. gattii* is therefore considered a primary pathogen, while *C. neoformans* is generally an opportunistic infection. The reason may be related to the level of environmental exposure, as immunocompetent individuals may have more exposure to *C. gattii* while outside working or enjoying outdoor activities. Host genetic factors and inherent resistance or susceptibility to cryptococcal infection may also play a role.[2]

Given that infection is typically via inhalation, disease due to *C. gattii* infection in animals most commonly presents as chronic rhinitis and pneumonia. Encephalitis, through direct invasion from the nasal cavity, or via hematogenous routes, may result in neurologic signs. Hematogenous spread may also result in widespread lesions involving many tissues. As mentioned above, cutaneous granulomatous lesions typically resulting from wound contamination may also occur, most commonly in cats.

Wild and domestic animals act as sentinels in the early detection of *C. gattii* infection before human cases are identified. Given the AVC case, white-tailed deer represent a new host species for *C. gattii* in North America. White tailed deer are non-migratory and generally exhibit minor seasonal movement within a geographic area, so this infection indicates that this fungus is present in Nova Scotia. In the past few decades, through cytology, biopsy and necropsy submissions to our laboratory, we have occasionally identified sporadic cases of cryptococcosis in dogs and cats as described in a February 2016 Diagnostic Update article. These cases have been presumed to be caused by *C. neoformans*, but it is possible that some or all were actually due to infection with *C. gattii*; genetic sequencing technology and Dr. Overy's expertise were not available to us at that time. Alternatively, this may herald a new emerging disease in Nova Scotia. Public health officials have been notified in Nova Scotia to include *C. gattii* infection on their list of differential diagnoses, as the pneumonia seen in human cases can mimic neoplasia or other infections. Their provincial public health laboratory also now has the capacity to test for this fungus.

If you encounter or suspect *Cryptococcus* infection in your patients, please feel free to contact me through the laboratory (902-566-0864) or e-mail (Andrea Bourque, abourque@upei.ca). We are very interested in these cases given their possible impact on animals and humans. To confirm the identity of this fungus we require fresh tissues or swabs for culture before we can proceed with the gene sequencing and fungal identification.

Acknowledgement: I wholeheartedly thank Drs. Scott McBurney and Dave Overy for sharing this case with me and all their good work on it. Thanks to Dr. Nicole Kaiser for the image.

References:

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4. Espinel-Ingroff, Kidd SE. Current trends in the prevalence of *Cryptococcus gattii* in the United States and Canada. *Infect Drug Resist*. 2015;8:89-97.
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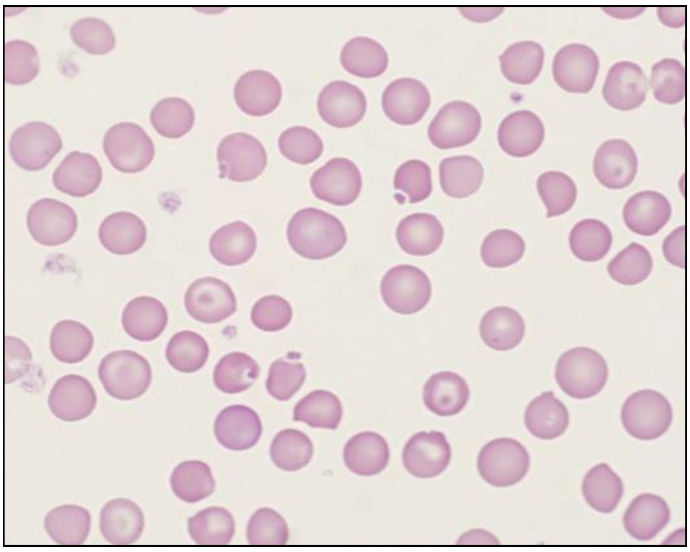
What's Your Diagnosis?



Blood smear from a dog. Wright-Giemsa, x 100 objective.

Ingestion of which of the following could fit with this blood smear from an anemic dog? See page 4 for the answer!

A. www.gsoextracts.com

B. www.theayurveda.org

C. www.21food.com

Time versus Concentration Dependent Antimicrobials

By Sandra McConkey, Veterinary Clinical Pathologist and Pharmacologist

The goal of prescribing any drug is to achieve the desired effect with minimal adverse effects. For antimicrobials, the aim is to either kill bacteria or sufficiently suppress bacterial growth while not adversely affecting the patient. This requires exposing bacteria to a high enough concentration of an antimicrobial drug for a long enough period of time. Antimicrobials can be categorized as time dependent, concentration dependent, or a combination of the two.

For time dependent drugs to be effective, the concentration must be above the minimum inhibitory concentration (MIC) >50% of the time. This rises to >80-90% of the time in immune suppressed animals. This is because time dependent drugs have minimal post antibiotic effects (PAE), meaning that their effect on bacteria is negligible once the concentration falls below the MIC. The ideal peak concentration for these antimicrobials is 2-4 times the MIC. Increasing the dosage to give a higher peak concentration doesn't improve the efficacy but could predispose to more adverse effects. Time dependent antimicrobials include beta-lactams, some macrolides, tetracyclines, trimethoprim sulfa combinations and chloramphenicol. A specific example is amoxicillin. The Veterinary Drugs Directorate (VDD) and Food and Drug Administration (FDA) approved label dosage for amoxicillin is 11mg/kg q12h, but in recent years veterinarians have been advised to give it q8h for less sensitive organisms or Gram negative bacteria such as *E.coli*. The most recent Plumb's Veterinary Drug Handbook (8th edition) goes even further and advises q8h amoxicillin for all canine urinary tract infections. Why? It is because amoxicillin is time dependent with some minor PAE against many organisms but no PAE for Gram negative organisms and *Streptococci*. Overall, it is now recognized that more frequent dosing improves the efficacy of amoxicillin.

Concentration dependent antimicrobials are most effective if

the peak concentration is 8-10 times the MIC. These drugs do not need to be above the MIC for an extended period of time because they often have significant PAE, meaning they continue to be effective for a period of time after the concentration falls below the MIC. These drugs are typically given at a high dose 1-2 times daily. A specific example is enrofloxacin. Initial dosage suggestions were at 5-20 mg/kg q24h or divided q12h, but the manufacturers now advise q24h because it is concentration dependent and requires a high peak for optimal efficacy. It may be tempting to divide the dose q12h in cats to lower the peak concentration to lessen the chance of retinal toxicity, but this decreases the efficacy. It is preferable to prescribe an alternative fluoroquinolone that is safer for cats such as pradofloxacin or marbofloxacin.

Many antimicrobials have a choice of dosages called a variable dosage range. It is important to avoid dosing below the range because the concentration may not be high enough or long enough above the MIC to be effective. This could also predispose to resistance. Veterinarians should be wary of going above the range because this could be toxic (e.g. aminoglycosides) or ironically, less efficacious (some fluoroquinolones). When treating an organism with intermediate sensitivity or an MIC at the breakpoint, the upper end of the dosage range should be used. The currently advised dosage range is used unless there is a reason to alter the dosage such as the location of the infection, altered pharmacokinetics due to concurrent medication or an underlying disease such as renal failure.

Fortunately, pharmaceutical companies and various pharmacokinetic studies provide veterinarians with suggested dosage ranges, but it is still important to remember that labeled doses may not reflect current literature. This is because it is costly for pharmaceutical companies to go through the process of having new label instructions accepted by the VDD or FDA. In the end, it is up to the veterinarian to choose the appropriate antimicrobial drug, stay current with changing protocols and choose the best dosage for each patient and infection.

References:

1. Giguere S. Antimicrobial drug action and interaction: an introduction. In: Giguere S, Prescott JF, Dowling PM, eds. *Antimicrobial Therapy in Veterinary Medicine*. 5th ed. Ames, IA: Wiley Blackwell; 2013:3-10.
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5. Plumb DC. *Plumb's Veterinary Handbook*. 7th ed. Ames, IA: Wiley-Blackwell; 2011.
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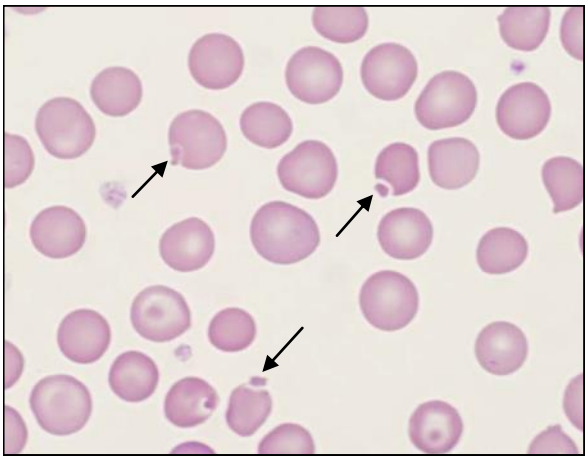
Answer to What's Your Diagnosis on page 3: Onions contain allyl propyl disulfide which causes oxidant damage to hemoglobin. The denatured hemoglobin structures are called Heinz bodies (arrows) and as they are removed from the circulation, anemia occurs. They are seen on routinely stained blood smears, especially when they extend from the cell periphery. However, they are even easier to see using New Methylene blue stain (Figure 1). In dogs, grapes (or raisins) cause kidney injury, while chocolate causes nervous signs including seizures. While both are severely toxic, neither cause Heinz bodies or acute anemia. While most dogs won't eat raw onions, owners can unknowingly give their pets a toxic dose by allowing them to eat pizza or other foods containing cooked onions. Luckily, most dogs recover from the anemia if they receive prompt veterinary care which may include blood transfusions.



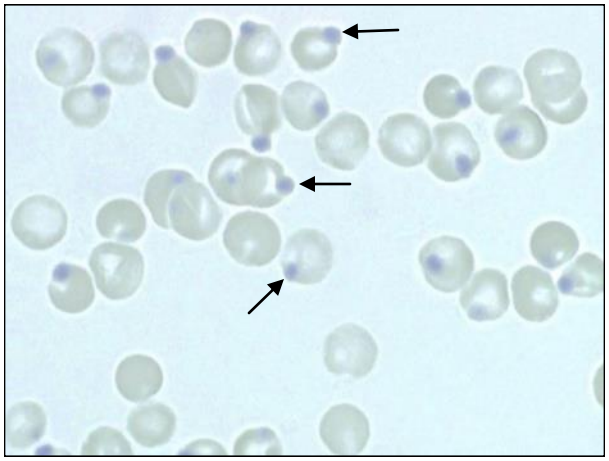


Figure 1: New Methylene Blue stained slide of a feline blood smear with Heinz bodies, x100 objective.

AVC Diagnostic Services Laboratory & Veterinary Teaching Hospital at the 2016 Atlantic Provinces Veterinary Conference

By Cornelia Gilroy, Veterinary Clinical Pathologist and Heather Gunn McQuillan, Director of the Veterinary Teaching Hospital

The Atlantic Veterinary College (AVC) Diagnostic Services Laboratory and the Veterinary Teaching Hospital (VTH) were represented at the annual Atlantic Provinces Veterinary Conference (APVC) in Halifax, Nova Scotia (Figure 1).

Figure 1: Our Diagnostic Services Laboratory and Veterinary Teaching Hospital booth at the APVC. Left to right: Ms. Nicole Rodgers and Dr. Andrea Bourque.

Anatomic pathologist, Dr. Andrea Bourque, and clinical pathologist, Dr. Cornelia Gilroy, represented the Diagnostic Services Laboratory. This provided a great opportunity to visit with colleagues and friends, as well as receive feedback on our services. The Diagnostic Services Laboratory was pleased to offer three draw prizes. Congratulations to our winners:

- First prize of two complimentary surgical biopsies: Ms. Charlotte Burkland from Cornwallis Veterinarians Ltd., Kentville, Nova Scotia.
- Second prize of two complimentary cytologies: Dr. Vanessa Scanlan from Fundy Veterinarians Ltd., Shubenacadie, Nova Scotia.
- Third prize of one complimentary complete blood count and chemistry profile test: Dr. Randy Hartt from Waterview Animal Hospital, Miramichi, New Brunswick.

Ms. Nicole Rodgers, the referring veterinarian liaison and Dr. Heather Gunn McQuillan, Director of the Veterinary Teaching Hospital, were available at the booth to meet and greet referring veterinarians and their staff. They were happy to address questions and concerns and had an electronic survey available for referring veterinarians with participants eligible for a prize draw. The prize (valued at $250) was for free admission to the first annual AVC Continuing Education (CE) Event, a gas card and Confederation Bridge pass. The winner was Dr. Janice Dimock.

The CE Event, sponsored by Zoetis, takes place Saturday, August 6, 2016, for veterinarians, registered veterinary technologists and animal health technicians. The program will feature three sessions, as well as an optional tour of the AVC Veterinary Teaching Hospital. Those who complete the course will receive credit for five CE hours. Dr. Chantale Pinard, DVM, MSc, Diplomate ACVO, will give a session entitled "*The 'red' eye and management of corneal ulcers*." Dr. Charlie Pye, DVM, DVSc, Diplomate ACVD, will give a presentation called "*Pathophysiology of itching and the use of Apoquel*." Ms. Andrea Jack, AHT, RVT, will present "*Blood donor programs: Being private practice prepared*." Ms. Andrea Jack coordinates the blood donor program at AVC and works in the Small Animal Hospital. For registration information, please visit upei.ca/avc/scee2016.

Thank you to everyone who invested time to stop by our exhibition booth. We look forward to seeing everyone again next year!

Further Assessment of Hypercalcemia in Dogs and Cats

By Cornelia Gilroy and Shelley Burton, Veterinary Clinical Pathologists

Hypercalcemia can occur due to a variety of underlying disorders or diseases as was reviewed in the article "Hypercalcemia in a Puppy" in the February 2016 Diagnostic Update issue. If the total calcium concentration is increased, the first step is to determine if the ionized calcium concentration is also high, as it is the biologically active form. The Diagnostic Services Laboratory can easily forward samples from your patient to Michigan State University for a diagnostic panel which includes ionized calcium, parathyroid hormone (PTH) and PTH related protein (PTHrp) concentrations.

This profile is recommended for cats and dogs with a persistent hypercalcemia to help differentiate some of the causes of hypercalcemia (Table 1). A 1 ml serum sample handled as anaerobically as possible is required for the ionized calcium and PTH, while a 0.5 ml sample of EDTA plasma (collected in a lavender topped tube) is needed for PTHrp. A 12 hour fast prior to sampling is preferred to minimize sample lipemia. Hemolysis should also be avoided as it can result in false positive PTHrp results. The samples should be centrifuged within 1 hour of collection and transported frozen to our laboratory. If indicated, 25-hydroxyvitamin D concentration can also be measured using a 0.5 ml serum or plasma (EDTA or heparinized) sample that is refrigerated or frozen.

If you have any questions concerning these tests please feel free to contact our laboratory staff (avcdiagnostics@upei.ca or 902-566-0860). We would be glad to answer your questions!

Table 1: Testing to investigate hypercalcemia in dogs and cats

Disorder	Ionized Calcium	Phosphorus	PTH	PTHrp	25-hydroxyvitamin D
Primary hyperparathyoidism	↑	↓ - WRI	WRI - ↑	WRI	WRI
Humoral hypercalcemia of malignancy	↑	↓	↓ - WRI	↑	WRI
Excess vitamin D	↑	WRI - ↑	↓ - WRI	WRI	↑
Chronic renal disease	Variable-mostly ↓	↑	WRI - ↑	WRI	↓ - WRI

WRI = within reference interval

New Racehorse Testing Bundle

The Diagnostic Services Laboratory is pleased to offer a new racehorse testing bundle in clinical pathology. This bundle is not exclusive to Standardbred horses but is available for all equines. This racehorse testing bundle includes the tests outlined in the following table.

If requesting this racehorse bundle, please submit both a lavender topped (EDTA) blood sample and non-additive red topped blood sample along with a completed requisition form for the patient stating "Racehorse Bundle" in the History block.

Please contact the laboratory (902-566-0860) for pricing or if you have any further questions about this new bundle.

Serum Chemistry	Urea Creatinine Creatine kinase (CK) Aspartate aminotransferase (AST) Gamma glutamyltransferase (GGT)
Endocrinology	Total thyroxine (T4)
Hematology	Automated cell counts Packed Cell Volume Protein Fibrinogen
Hematology-optional add on for an additional fee	White blood cell differential Red cell evaluation

Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

Here are some recent happenings in the Diagnostic Services Laboratory:

- Welcome to Nicole Bishop, our new hematology technologist, who began her position in May 2016.
- Several members of Diagnostic Services and the Veterinary Teaching Hospital travelled to Halifax in April to participate in the Atlantic Provinces Veterinary Conference (please see full article on page 5).
- The Canadian Animal Health Laboratorians Network (CAHLN) 15th annual meeting was held in Charlottetown, Prince Edward Island from June 5th - 8th and hosted by representatives from Diagnostic Services laboratory. The theme for the CAHLN 2016 Annual Meeting was "Collaboration in Veterinary Laboratory Diagnostics and Animal Health". There were 118 attendees at the meeting. Congratulations to Dr. Alfonso Lopez, who was awarded The Laboratorian of the Year award at this conference.
- Dr. Cornelia Gilroy, Veterinary Clinical Pathologist, was awarded the 2015-2016 University of Prince Edward Island Faculty Association Hessian Merit Award for Excellence in Teaching.
- Congratulations to Matt Saab, bacteriology technologist, who successfully defended his Master of Science thesis entitled "Methicllin-resistant *Staphylococcus pseudintermedius* in Atlantic Canada: Epidemiology and Culture Methods".
- Congratulations to Dr. Melanie Buote, anatomic pathologist, who successfully defended her PhD thesis entitled "Microscopic and metabolomic investigation of *Hematodium* sp. infections in Newfoundland snow crabs, *Chionoecetes opilio*".
- Congratulations to Ellen McMahon, (Figure 1) the recipient of the 2016 Atlantic Veterinary College Staff Merit Award. Ellen is a long time employee of the Diagnostic Services Laboratory who is very deserving of this award for her attention to detail, cheerful attitude and diligent work ethic, in addition to other reasons too numerous to list!

Figure 1: Ellen McMahon (in the middle), with two of her nominators (left to right; Dr. Cornelia Gilroy and Dr. Shelley Burton), is the recipient of the 2016 Atlantic Veterinary College Staff Merit Award. Ellen is in costume for the jungle themed Atlantic Veterinary College Summer Social.

Staff Focus

Jan Giles

By Cornelia Gilroy, Veterinary Clinical Pathologist and Anne Muckle, Veterinary Clinical Bacteriologist

Jan Giles has been a cornerstone in the Atlantic Veterinary College (AVC) Diagnostic Services Bacteriology Laboratory for many years. She is well known for her generous smile, great sense of humor, hard work and willingness to answer questions from students, colleagues and clients.

Originally from Truro, Nova Scotia, Jan began her post-secondary education in the Biology Laboratory Technology Program at the Nova Scotia Agricultural College (NSAC). She subsequently completed 2 years in a Bachelor of Science (BSc) degree program at the NSAC and then finished her BSc in microbiology at the University of Guelph in 1987. Jan was introduced to working with fish in Ontario and then came to the University of Prince Edward Island (UPEI) to pursue a Master of Science (MSc) degree investigating fish bacterial pathogens. After completing her MSc degree in 1992, Jan continued working at UPEI. It was while at UPEI that Jan met her husband, Dr. Don Rainnie, a fish veterinarian. They married and over the next 10 years, Jan primarily focused her energies on raising their now grown sons, Nathan and Aaron.

Jan's love of learning had her enroll in a Bachelor of Education (BEd) program at UPEI in 2003. After completing this in 2005, Jan was hired to investigate alternate methods of teaching statistics. She also returned to the field of microbiology by assisting Dr. Jeff Lewis in the AVC Department of Pathology and Microbiology with teaching and research. Jan began working with Diagnostic Services in 2006 and became the Lead Bacteriology technologist in 2008.

Jan has an important role as our aquatics bacteriologist. Her expertise and passion for identifying fish bacterial pathogens is highly valued by our clients. Besides her diagnostic work, Jan publishes scientific articles and gives presentations at local and international fish conferences. Her other roles are many, including routine mammalian diagnostic bench work, responsibilities as Lead Technologist, assisting with teaching bacteriology to undergraduate and graduate students, and participating in the AVC Vet Camps and the AVC Summer Academy sessions.

When not working, Jan enjoys spending time with her family, country living, reading, gardening and sampling local wines. She has volunteered with several groups over the years, including the PEI Youth Chess Association, the Singing Strings Youth Orchestra and organizing Scholastic Book club events in schools. She has a dedication for life-long learning, as evidenced by her years of formal studying. This serves her well as each day brings diagnostic challenges and Jan has the ability to make microbiology interesting and understandable for others. She is an excellent microbiologist who rises to these challenges daily as she provides exemplary service to the clients of Diagnostic Services!

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.