Diagnostic Update

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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 12:00 pm

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In this Issue:

Sporadic cases of Cryptococcosis in the Maritimes

By Shannon Martinson, Veterinary Anatomic Pathologist

Deep mycotic infections in animals and humans are mainly caused by dimorphic fungi of the following genera: *Cryptococcus*, *Blastomyces*, *Histoplasma* and *Coccidioides*. While these fungi are generally considered nonexistent in our region, we occasionally identify them in histologic and cytologic samples. Cryptococcosis occurs worldwide affecting a wide variety of mammalian species. Over the past several years, we have seen sporadic cases of cryptococcosis occurring in pets from New Brunswick, Nova Scotia and Prince Edward Island, including 2 cases this year (Table 1). Over the same time period, we have seen only 2 cases of blastomycosis, both with a history of travel to endemic regions in western Canada, and no cases of histoplasmosis or coccidioidomycosis.

Table 1: Biopsy and necropsy cases of cryptococcosis, AVC Diagnostic Services Laboratory, 1990-2015.

Year	Species	Signalment	Organ/system involved	Case origin
1992	Feline	Not provided	Nasal	Saint John, NB
1999	Canine	7 year old, FS, Doberman Pinscher	Disseminated	New Minas, NS
2000	Feline	8 year old, FS, DSH	Nasal	PE
2001	Feline	6 year old, MC, DSH	Nasal	Amherst, NS
2004	Feline	13 year old, FS, DSH	Nasal	Halifax, NS
2004	Canine	3 year old, MC Cocker Spaniel	Disseminated	NB
2015	Feline	7 year old, MC, DSH	Skin and LN	Baddeck, NS
2015	Feline	6 months old, FS, DLH	Tongue	New Glasgow, NS

There are more than 30 species of *Cryptococcus* but the majority of clinical cases in companion animals are caused by those in the *Cryptococcus neoformans – Cryptococcus gattii* species complex. These fungi exist in the environment in filamentous or yeast forms; *C. neoformans* is commonly associated with pigeon feces while *C. gattii* is more commonly found in the soil or decaying hollows of certain types of trees, including eucalyptus trees. While *C. gattii* has historically been restricted to tropical and subtropical locations, this species emerged in British Columbia in 1999. It causes disease in both humans and animals and is now considered endemic in that region.

While both humans and animals may be infected by organisms in the *Cryptococcus neoformans – gattii* complex, the disease is not considered zoonotic or contagious. This is because it is acquired through environmental exposure to aerosolized fungal spores or desiccated yeasts and is not transferred from host to host. Most often the spores, which are easily dispersed by air flow, are inhaled and initiate infection in the upper respiratory system. Less often, infection is gained through ingestion of yeasts or cutaneous implantation. In humans, infections with *C. neoformans* occur most commonly in immunosuppressed individuals while *C. gattii* may infect immunocompetent hosts. Both agents may cause infection in immunocompetent animals. These organisms exist as yeasts in mammalian host tissues.

These fungi may cause infection in a variety of species including humans, cats, dogs, pigs, cattle, birds, ferrets, horses, porpoises and reptiles. In cats and dogs, they may colonize the nasal passages asymptomatically. Within domestic species, cats are most commonly affected and typically develop upper respiratory infections with sneezing, nasal discharge, snorting and dyspnea. Occasionally polyps may extrude from the nose and cutaneous nodules, often on the bridge of the nose, may occur. Less commonly pneumonia, encephalitis and systemic disease are reported. Indeed, of the cases seen in our laboratory (Table 1), most were in cats (6/8 cases) and rhinitis was the most common presentation (4/6 cases). We have also seen skin and lymph node involvement and most recently, glossitis. No sex or breed predilections have been shown and, despite the route of infection, *Cryptococcus* infections are occasionally reported in indoor cats. There are conflicting reports of the involvement of feline leukemia virus and feline immunodeficiency virus as predisposing factors for this disease. In dogs, cryptococcosis is more often a life threatening systemic illness. In the two cases diagnosed in our laboratory, the dogs had nonspecific clinical signs including cough, vomiting and seizures, and were ultimately humanely euthanized. Granulomatous lesions containing numerous yeast were identified in most organs (including the brain) in both cases.

A diagnosis of cryptococcosis is typically made via the detection of yeasts in cytological samples (Figure 1A), often from nasal swabs, body fluids or lymph node aspirates, or on histopathologic examination of tissue obtained via biopsy or necropsy. The yeasts are identified based on morphology. They range in size from 4 to 15 µm and have a characteristic thick polysaccharide capsule seen on routine staining as a clear space around the fungus (Figure 1A and 1B). The organisms exhibit narrow based budding and are positive with Periodic Acid Schiff (PAS) reaction (Figure 1C) and mucicarmine staining (Figure 1D). While most other yeasts fail to stain with mucicarmine, *Blastomyces* may stain weakly positive. Definitive diagnosis with speciation requires fungal culture and further molecular testing done at a referral laboratory. Serologic antigen tests also can be performed on serum or body fluids (including urine); serial serologic testing may be helpful in monitoring response to medical therapy.

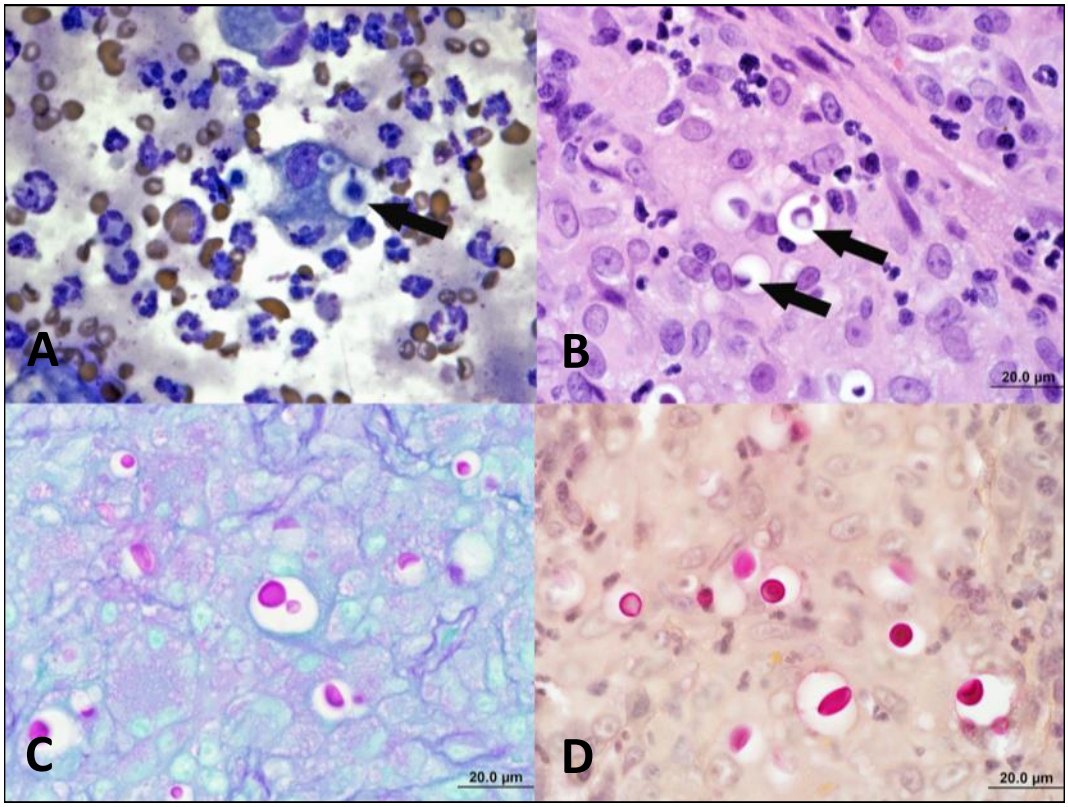


Figure 1: Diagnostic samples containing *Cryptococcus* species. (**A**) Cytology, fine needle aspirate of a nasal mass from a cat with cryptococcosis. Narrow based budding and the characteristic thick capsule are evident (arrow). Diff Quick, x 100 objective. (**B**) Histopathology, mass from the tongue of a cat with cryptococcosis. Pyogranulomatous infiltrates are evident surrounding yeast (some budding) with a thick clear capsule (arrows). H&E, x 100 objective. The fungus stains positively with PAS (**C**) and mucicarmine (**D**) stains. x 100 objective.

While rare in our region, cryptococcosis remains an important differential for respiratory and cutaneous disease in cats and may occasionally cause fatal systemic infections in dogs in the Maritime provinces.

References:

1. Vorathavorn VI, Sykes JE, Feldman DG. Cryptococcosis as an emerging systemic mycosis in dogs. *J Vet Emerg Crit Care.* 2013;23(5):489-497.
2. Pennisi MG, Hartmann K, Lloret A, et al. Cryptococcosis in cats. ABCD guidelines on prevention and management. *J Fel Med Surg.* 2013;15:611-618.

MIC Testing and Reporting Explained

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

We recently switched to minimum inhibitory concentration (MIC) testing in order to help veterinary practitioners select the optimal antimicrobial therapy for their patients. We perform MIC testing using a semi-automated system that ensures standardization and quality for each result. Our testing conforms to the Clinical Laboratory Standards Institute (CLSI) guidelines for antimicrobial susceptibility testing (AST) for bacteria isolated from animals.

What are MICs and what do these numbers mean?

The MIC is the lowest concentration of a specific antimicrobial required to kill or inhibit the *in vitro* growth of a particular bacterial organism. MICs are reported as a value in µg/mL for each antimicrobial tested. These values are interpreted and reported as susceptible (S), intermediate (I), or resistant (R). A breakpoint is the MIC value that defines these interpretative criteria. These interpretations and breakpoints are specific for each antimicrobial-organism combination, and in some cases they are host or body site specific (Table 1). CLSI determines and regularly reviews interpretations and breakpoints for certain bacteria and drugs.

Table 1: MIC and breakpoint data for *Escherichia coli* isolated from a dog.

Antimicrobial	MIC (µg/ml)	Body Site	Breakpoints (µg/ml)		
			S	I	R
Amoxicillin-Clavulanic Acid	2	Urine	≤8		>8
		Skin, soft tissue	≤0.25	0.5	≥1
Enrofloxacin	0.5	All sites	0.5	1-2	≥4

How do I use MIC values to decide which antimicrobial(s) to treat with?

An important feature of the MIC report is that it allows you to select the most susceptible drug for the organism being tested. A drug with an MIC farthest from its susceptibility breakpoint means that the bacteria is more susceptible to this drug than another drug whose MIC is closer to its susceptibility breakpoint. This is illustrated in Table 1. In this example, when only bacterial factors are considered, amoxicillin-clavulanic acid is the better choice if the bacteria were cultured from urine. Although *Escherichia coli* has a lower MIC with enrofloxacin, the MIC is at the breakpoint, while the amoxicillin-clavulanic acid MIC is 2 dilutions from the breakpoint. On the other hand, if this was a skin culture, enrofloxacin is the better choice because amoxicillin-clavulanic acid is resistant at an MIC of 2 µg/ml.

Breakpoint data are not currently on our MIC reports, but we are working to have this information included. In the meantime, you can use the *In vitro* Antimicrobial Susceptibility Testing Charts in the book, *TARGET – The Veterinary Antimicrobial Reference Guide to Effective Treatment, March 9, 2015, 5th Edition*, which is available in print or may be downloaded free to your Apple or Android smart phone.

Why are some antimicrobials not on our reports?

Each veterinary diagnostic laboratory makes decisions about which drugs to include on MIC reports and in what situations. Although there is no standard approach in veterinary medicine, we are using selective reporting for certain bacteria and antimicrobials, such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in which secondary drugs are only reported if an organism is resistant to primary drugs. CLSI interpretative guidelines are not always available for a particular drug or

organism-animal species combination. There are a number of veterinary drugs that do not have veterinary specific break-point data, so in some cases, human breakpoints will be used. Certain drugs are class representative drugs tested to predict the activity of other drugs in the same class. For example, ampicillin is used to test for amoxicillin, and cephalothin is the class representative for first generation cephalosporins (except cefazolin). Therefore, although you may not see a result for a specific drug, its susceptibility can be inferred from other results provided.

Why are MIC results not given for all organisms isolated from a clinical specimen?

It is widely recognized that there is a need for improved antimicrobial use practices to reduce bacterial antimicrobial resistance (AMR). As diagnosticians and clinicians, we have a responsibility to improve antimicrobial use practices in veterinary medicine.[2, 3] We must remember that not all animals that are ill have bacterial infections and that not all bacterial infections require treatment with antimicrobials.[3] Diagnostic laboratories are encouraged to withhold reporting of isolates that are deemed clinically irrelevant based on the bacterial species and the site of infection (such as *Enterococcus* and *Corynebacterium* species) and to use selective drug reporting to optimize animal care and foster antimicrobial stewardship. These decisions are determined case-by-case by the veterinary clinical bacteriologist, and may require discussion with the submitting veterinarian and other diagnosticians. The quality of culture results and their interpretation requires the submission of a good quality specimen and a completed submission form with a clinical history (refer to our newsletter article on specimen submission http://avc.upei.ca/files/avc/August_2014.pdf).

How can I learn more about my role in antimicrobial stewardship?

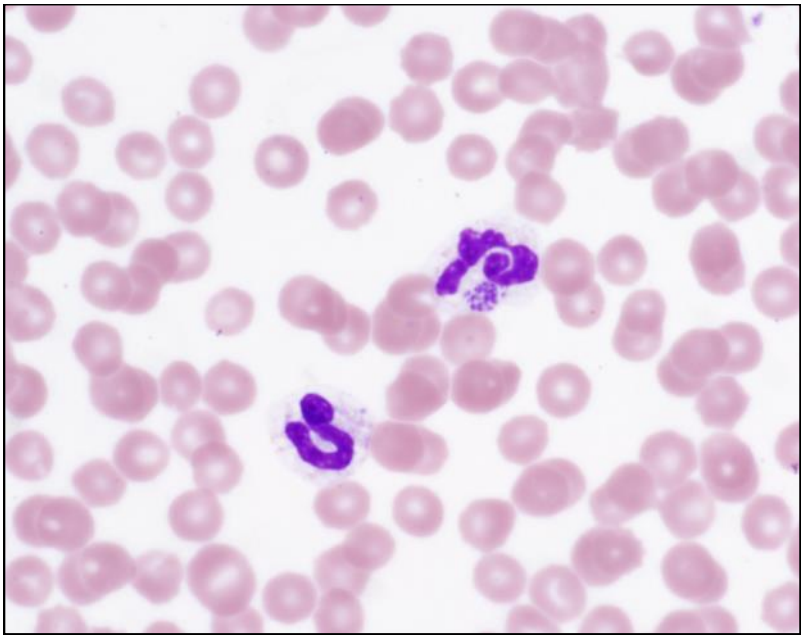
We strongly recommend the American College of Veterinary Internal Medicine (ACVIM) consensus statement on antimicrobial use in animals.[3] It provides recommendations on antimicrobial use and balancing effective therapy while minimizing the development of AMR in bacteria. For small animal practitioners, we highly recommend the TARGET book.[1] It provides extensive information and easy to use tables on drugs and dosing strategies for 24 microbial drugs against most of the common pathogens in dogs and cats.[1]

The Atlantic Veterinary College Diagnostic Services Bacteriology Laboratory welcomes your dialogue and input in the processing of your clinical specimens. Please contact us for further information on MIC testing or the interpretation of susceptibility results (902-566-0821).

References:

1. Aucoin D. *TARGET - The Antimicrobial Reference Guide to Effective Treatment*. 5th ed. North American Compendiums, Inc. 2015.
2. Antimicrobial Use in Animals - Position Statement. Canadian Veterinary Medical Association. November 14, 2014. Available at: http://www.canadianveterinarians.net/documents/antimicrobial-use-in-animals. Accessed: June 1, 2015.
3. Weese JS, Giguère S, Guardabassi L, et al. ACVIM consensus statement on therapeutic antimicrobial use in animals and antimicrobial resistance. *J Vet Intern Med.* 2015;29:487-498.

What's Your Diagnosis?



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

What is your diagnosis?

See page 8 for the answer.

All the Best to our Retirees!

By Cornelia Gilroy and Shelley Burton, Veterinary Clinical Pathologists

After more than 25 years of service at the Atlantic Veterinary College (AVC), Lorraine Lund, Linda Ruschkowski and Ramona Taylor have retired from their positions with the Diagnostic Services Laboratory.

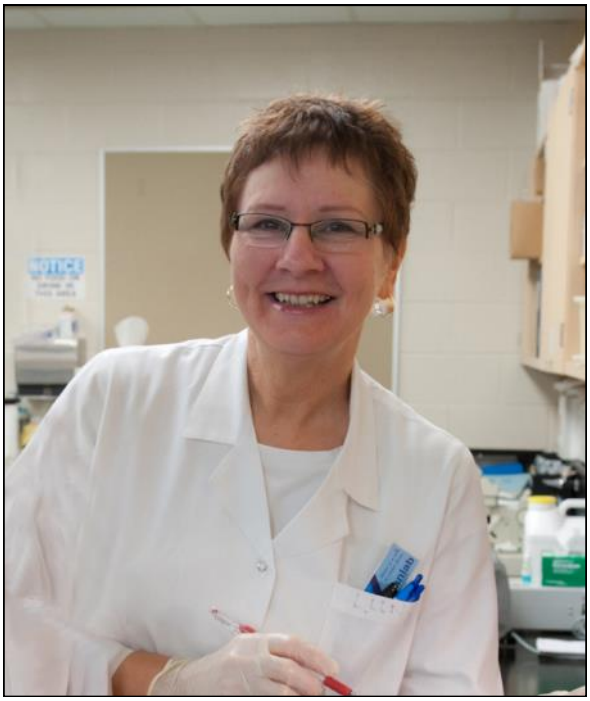


Figure 1: Lorraine Lund

Lorraine Lund retired in July 2015 from her position as a bacteriology technologist which she had worked at since 1987 (Figure 1). Her duties included benchtop identification of organisms, sensitivity testing and assisting with teaching of 4th year veterinary students on rotations. Lorraine has many other interests such as genealogy, gardening, traveling and reading historical fiction as well as spending time with her 2 grandchildren. Lorraine also enjoys a fine glass of wine, especially red wine made by her husband, Chuck.

Linda Ruschkowski retired in March 2015 from her position as chief hematology technologist (Figure 2). Linda began working in Diagnostic Services in 1987. Her duties included processing samples for cytology and hematology, blood smear evaluation, cross-matching and hemostasis testing. Linda and her husband Dan, along with their beloved pets, moved back home to Saskatchewan. Linda is currently working at Assiniboia Union Hospital in Saskatchewan as a medical laboratory technologist. When not working, Linda enjoys sewing for herself and others. She is a highly skilled seamstress whose skills are often in demand making beautiful wedding gowns or historical costumes.

Figure 2: Linda Ruschkowski

Figure 3: Ramona Taylor

Chief histology technologist, Ramona Taylor, retired from her position in January 2016 after working at the AVC since 1986 when it first opened (Figure 3). Her duties included histologic processing and special stains – both of which she spent time perfecting in order to produce the best quality slides possible! Ramona and her husband, Errol, have 3 grown daughters, Alana, Carolyn and Ellen, as well as 4 grandchildren. She enjoys independent and foreign films, gardening, reading and travelling. In addition, Ramona is a skilled soprano and is considered one of PEI's best gospel music singers.

We would like to wish Lorraine, Linda and Ramona all the best with their retirements from the AVC Diagnostic Services Laboratory. They each contributed a great deal in their areas of specialization. Happy retirement!

Hypercalcemia in a Puppy

By Cornelia Gilroy and Shelley Burton, Veterinary Clinical Pathologists

An interesting case was recently seen at the Atlantic Veterinary College (AVC). It involved a 4 month old male Staffordshire terrier dog with severe skin disease which had resulted in almost complete alopecia and pyoderma. The puppy was depressed and weak upon presentation. Apart from the changes attributable to his young age, significant abnormalities on serum chemistry included a marked hypernatremia, a mild increase in urea and a marked hypercalcemia (Table 1). The hypernatremia and increased urea concentration were attributed to dehydration.

Table 1: Serum total and ionized calcium concentrations.

Day	Serum Calcium (mmol/L) Adult Reference Interval: 2.02—2.91 mmol/L	Ionized Calcium (mmol/L) Adult Reference Interval: 1.01—1.46 mmol/L
1	4.01	
2	4.28	
3	4.49	1.50
6	4.12	2.13, 2.25
7	3.53	1.88
8	3.31	1.77
9	3.17	
10	2.94	

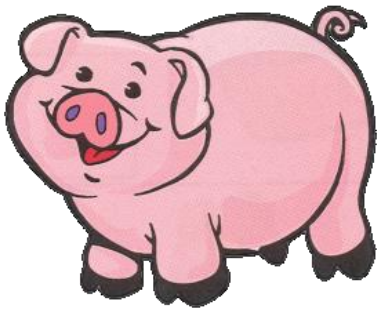
A skin scraping revealed *Demodex* mites and low numbers of yeast organisms and bacteria along with inflammatory cells. Additional testing at an external laboratory included assessment of concentrations of parathyroid hormone (PTH), PTH related protein (PTHrP), ionized calcium and 25-hydroxyvitamin D (Table 2). Lymph node aspirates revealed reactive hyperplasia while a skin biopsy provided a diagnosis of severe chronic pyogranulomatous dermatitis with intrafollicular mites consistent with *Demodex* species. The puppy responded to therapy with antimicrobial and antiparasitic drugs, as well as medicated shampoos. After therapy commenced on days 1 and 2, the serum calcium concentration began to decrease.

Table 2: Additional testing on day 7.

Analyte	Result	Reference Interval
Ionized calcium	1.68 mmol/L	1.25 - 1.45 mmol/L
PTH	0.3 pmol/L	0.5 - 5.8 pmol/L
PTHrP	0.0 pmol/L	0.0 - 1.0 pmol/L
25-hydroxyvitamin D	37 nmol/L	60 - 215 nmol/L

The PTH and PTHrP concentrations did not support a diagnosis of primary hyperparathyroidism and humoral hypercalcemia of malignancy, respectively. The slightly low PTH concentration was considered an appropriate response to the hypercalcemia. The low vitamin D (25-hydroxyvitamin D) ruled out excessive dietary intake of vitamin D. As the serum calcium concentration decreased as the skin disease resolved, it is believed that the hypercalcemia was due to a combination of young age and granulomatous inflammation. This case was interesting because even though calcium concentrations may be slightly higher in puppies than adult dogs, this puppy had such a high value that further investigation was warranted.

Hypercalcemia can occur due to a variety of underlying disorders or diseases. Some of the common or important differentials can be remembered with this fun mnemonic HOG IN YARD:



Hog in Yard

H: Primary hyperparathyroidism, hemoconcentration, hyperproteinemia

O: Osteolytic lesions – such as osteomyelitis

G: Grape ingestion – if acute renal failure occurs
Granulomatous disease – macrophagic inflammation, such as fungal disease

I: Idiopathic – often considered the top reason for hypercalcemia in cats

N: Neoplasia – also called humoral hypercalcemia of malignancy

Y: Young – young animals can have this due to bone remodeling but it is not common (unlike increased P)

A: Addison's disease – hypoadrenocorticism (not in every case)

R: Renal disease – more common in horses as the kidneys usually excrete a large amount of calcium. Not as common to see in dogs and cats – maybe 10-15% of renal failure cases.

D: Vitamin D toxicosis – from administration, accidental ingestion or cholecalciferol rodenticide toxiciosis.

Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

Here are some recent happenings in the Diagnostic Services Laboratory:

- We wish all the best to Lorraine Lund (bacteriology technologist), Linda Ruschkowski (hematology technologist) and Ramona Taylor (histopathology technologist) who retired after many years of service at the AVC (please see full article on page 5).
- Congratulations to Dr. Shelley Burton on being elected President of the American Society for Veterinary Clinical Pathology (ASVCP) at the ASVCP annual general meeting in October 2015 in Minneapolis, Minnesota.
- Dr. Cora Gilroy, Veterinary Clinical Pathologist, was awarded the 2015 Zoetis Carl J. Norden Distinguished Teacher Award, the highest teaching award given by North American veterinary colleges.

Staff Focus

Dr. Barbara Horney

By Shelley Burton, Veterinary Clinical Pathologist

Dr. Barbara Horney is well known as a mainstay of excellent clinical pathology work at the Atlantic Veterinary College (AVC) for many years. Barb grew up in Guelph, Ontario, and was surrounded by veterinary medicine her entire life, since her father, Dr. Donald Horney, was a large animal surgeon at the Ontario Veterinary College (OVC). As they say, the "apple doesn't fall far from the tree"; Barb entered veterinary school at the OVC, becoming increasingly intrigued with clinical pathology. Her parents were definitely proud when she graduated with the gold medal for the highest academic average of her veterinary class in 1982. Barb then entered a PhD program under the supervision of Dr. Ted Valli, a renowned hematopathologist, and graduated in 1987. Following an enjoyable time at the diagnostic laboratory in Kemptville, Ontario, she moved to PEI to join the AVC in 1988, working with David Honor in the early development of courses and the diagnostic laboratory. She became a Diplomate of the American College of Veterinary Pathologists in 1990.

Barb has a passion for ethics and integrity in research and veterinary professionalism, and has funnelled that interest into lectures and committee work both locally and farther afield. She is past President of the Society for Veterinary Medical Ethics and currently serves on the University of PEI Ethics Board. Her leadership positions have included the presidency of the PEI Veterinary Medical Association and the Canadian Association of Veterinary Pathologists. She currently is representative for the AVC and the University of Montreal on the Canadian Veterinary Medical Association Council.

The AVC clinical pathology group and our diagnostic laboratory have benefitted greatly from Barb's many attributes, including her quick mathematical mind (she is a human calculator!), kind support of colleagues and students and numerous contributions to improving courses and laboratory quality. Barb is our resident Lyme disease and leptospirosis expert and always seems to be the one on duty when interesting infectious disease cases come through! She is always in good humor and has an infectious laugh; both are much appreciated during busy work days. Barb loves a good debate and is astonishingly knowledgeable about widely varying aspects such as unusual pathologic diseases, world events and movie star trivia.

Barb has many interests outside of veterinary medicine. Along with her husband, Carl, she enjoys time spent in the PEI woods on horseback and on the water in canoes or kayaks. They have 2 grown children, Sam and Ellie, who are forging successful careers of their own in engineering and science. Barb is an avid reader and CBC radio listener….and in the other meaning of that abbreviation, an avid CBC data interpreter!

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

Answer to What's Your Diagnosis on page 4: Neutrophil containing an *Anaplasma phagocytophilum* morula. *Ehrlichia ewingii* morulae also have a similar appearance and cannot be differentiated visually from *A. phagocytophilum* morulae.