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/diagnosticservices

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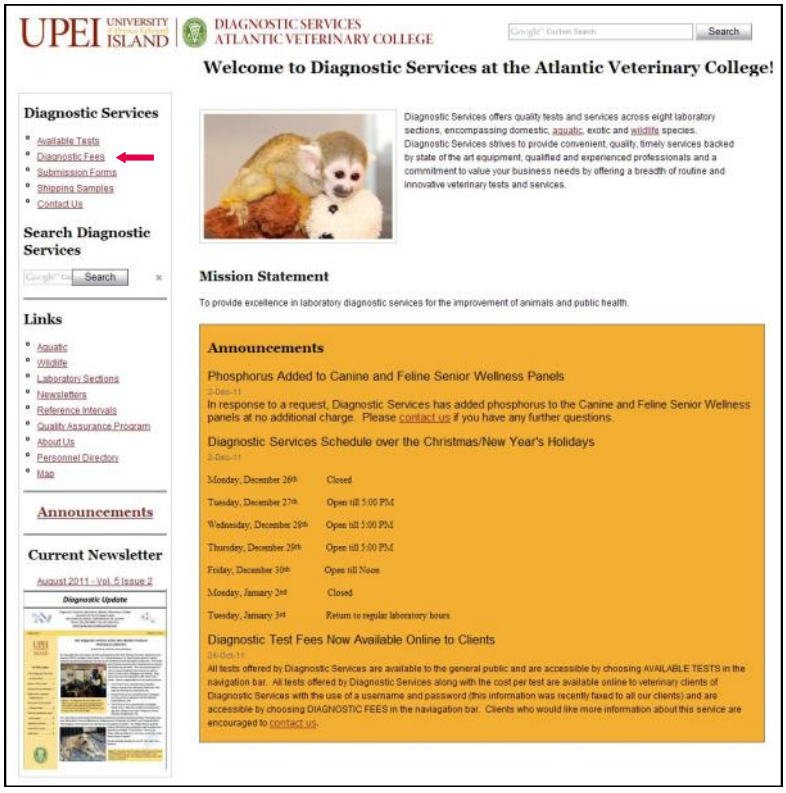
Enhanced Website Services

By Cornelia Gilroy, Veterinary Clinical Pathologist

What can our website offer you? Here are some great new features!

- Diagnostic test fees are available online (arrow in Figure 1) that are password protected. Please contact us (902-566-0863 or avcdiagnostics@upei.ca) for further information or to obtain a password.
- A searchable available test guide that includes test name, testing days, turn around times, required sample as well as sample handling and storage information.
- Downloadable submission forms that can be printed and completed by hand or PDF forms that can be typed directly into, saved and then printed.
- Keep up to date with the announcements from our laboratory.
- Guidelines and tips for shipping samples to the laboratory so that they arrive intact!
- Detailed information pertaining to the various laboratory sections.
- Present and past newsletters from Diagnostic Services.



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.

Double Trouble: Investigation of Two Tumors in a Pet Gerbil

By Heather Fenton, Wildlife Anatomic Pathology Resident

A three-year-old female gerbil (*Meriones unguiculatus*) presented to the Atlantic Veterinary College (AVC) Veterinary Teaching Hospital with a large non-healing wound on the left leg that was affecting weight bearing.

The cytologic diagnosis based on fine needle aspiration was compatible with a carcinoma (Figure 1). The owners elected euthanasia and the gerbil was submitted for necropsy. Post-mortem examination revealed a subcutaneous mass extending from beneath the left mandible to the middle of the rib cage. Histologic evaluation of this mass was consistent with a poorly differentiated malignant neoplasm (Figure 2) with evidence of metastasis to the lung. Further evaluation included a Fontana-Masson stain (for melanin) and immunohistochemistry for cytokeratin (to identify epithelial tissue) and vimentin (to identify mesenchymal tissue). The Fontana-Masson result was negative and the immunohistochemical result was positive for cytokeratin 19 and negative for vimentin. The other post-mortem finding was that the right ovary was approximately 10 times the diameter of the left ovary. Histologic evaluation of right ovarian tissue was consistent with a luteoma.

Neoplasia is a common finding in laboratory gerbils with approximately 25-40% developing neoplasia by 2 years of age.[1] Benign tumors of the ovary (usually granulosa cell and thecal tumors) are most common.[2, 3] It was difficult to determine the cell of origin of the subcutaneous tumor due to the poorly differentiated cells, but the immunohistochemical staining was consistent with a carcinoma, possibly salivary or mammary in origin. Salivary gland tumors are rare in domestic animals (0.17% of tumors in dogs and cats).[4] Mammary gland tumors of gerbils are also rare despite the common occurrence in other rodent species.[2] Other possibilities include a poorly differentiated squamous cell carcinoma,[2] melanoma[2, 5] or basal cell carcinoma (trichoblastic carcinoma).

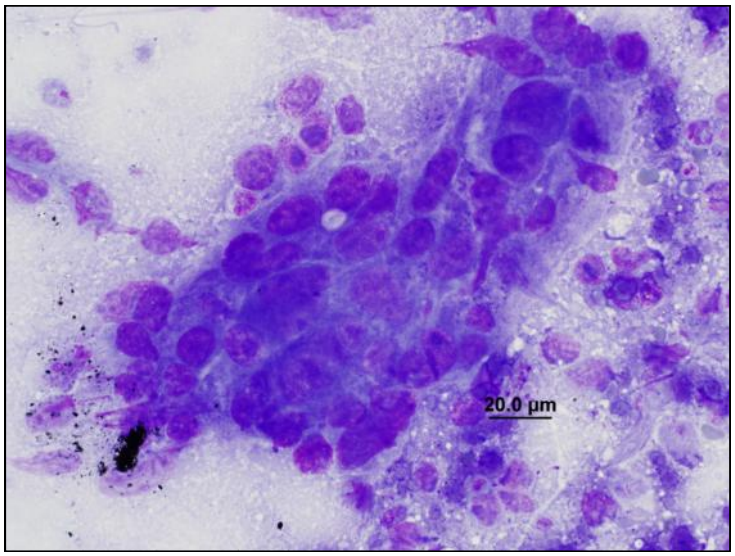


Figure 1: Fine needle aspirate smear of epithelial population with malignant criteria. Wright-Giemsa.

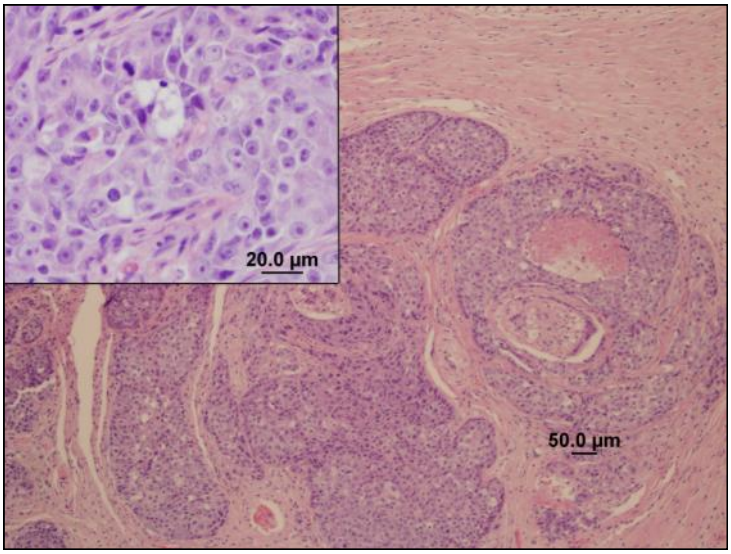


Figure 2: Histologic section of subcutaneous malignant tumor. H&E.

The high grade malignant tumor with pulmonary metastasis seen in this case would presumably have had a poor prognosis. This case is a good reminder of the importance of performing fine-needle aspirates on what appear to be non-healing wounds. It also highlights recent advances in immunohistochemistry offered at the AVC Diagnostic Services to further characterize tumors.

Acknowledgements:

Special thanks to Meghan Woodland, Marion Desmarchelier, Cornelia Gilroy, María Forzàn, Kathy Jones, Soraya Sayi, Aimee Elson, Letitia Chow and Sarah Mouri for their assistance with this case.

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"Food for Thought" - Re-emerging Diseases in Organic Free-range Farming

By Paul Hanna, Veterinary Anatomic Pathologist

In recent years, we have seen an increasing segment of society shift to buying free-range and naturally raised meat, eggs and dairy products. This movement is undoubtedly a response to increased public concern about several contentious issues around intensive livestock rearing methods. Perhaps the most important of these are the widespread use of antibiotics in animal feeds and animal welfare issues, such as restricted living space for chickens (battery caged hens) and sows (gestation crates). While these concerns are real, the consequences of switching to older style organic and/or free-range farming, particularly with regard to disease issues, are not well known by the public.

The following are examples of diseases recently seen in diagnostic laboratories in animals submitted from organic or free-range farms:

Case 1 – Pigs with parasitic bronchitis and brochiolitis due to infection with *Metastrongylus*: These nematodes require an earthworm as an intermediate host and infections are therefore rare in conventional barns where the pigs are reared entirely indoors. Control is difficult if pigs have outdoor access, as infested earthworms can persist in infected soils for up to 4 years.

Case 2 – Pigs with wasting disease and pneumonia due to porcine circovirus type 2 and parasitic hepatitis ("milk spot liver") due to *Ascaris suum*: While both of these diseases occur in conventionally reared swine, the incidence has been greatly reduced by effective vaccination for porcine circovirus type 2 and use of anthelmintics and good hygiene practices in modern pig barns. Control of ascariasis is difficult in free-range systems as soil is difficult to decontaminate and the eggs may persist over the winter. Additionally, any cattle allowed access to areas where swine have shed *Ascaris suum* are at high risk for a serious interstitial pneumonia caused by aberrant migration of the larvae of this nematode.

Case 3 – Pigs with chronic arthritis due to infection with *Erysipelelothrix rhusiopathiae*: Erysipelas infections are now relatively rare in conventionally raised pigs as effective vaccines have been available for many years.

Case 4 – Turkeys with histomoniasis ("blackhead"): Each year, we see numerous cases of this in free-range turkeys and even in chickens, which have some natural resistance to this disease. These infections are rare in conventionally raised poultry as they are not exposed to earthworms which harbour the *Heterakis gallinarum* nematode larvae containing the causative protozoa, *Histomonas meleagridis*. Heterakis eggs, which also contain *Histomonas* organisms, are highly resistant, remaining viable in the soil for many years.

Although not yet seen in our laboratory, there is preliminary data suggesting re-emergence or increased incidence of significant foodborne pathogens in swine raised outdoors without antimicrobials.[1]

So if some of the natural, organic, or free range farming practices result in increased disease and mortality, do they really contribute to an overall improvement in animal welfare and a healthier food supply? Perhaps the answer lies somewhere between the poles of more intensive conventional production and completely organic free-range farming. The European Union has lead the way in dramatically reducing the use of antibiotics in livestock production by eliminating use of antimicrobials as growth promoters. California has put in place minimal space requirements for poultry and swine rearing. Since we know that biosecure barns and effective vaccines dramatically reduce disease prevalence with resulting healthier animal products, are they necessarily bad just because they are modern? It is important that consumers realize that switching to an older style production system may have some unintended consequences, especially with regard to re-emerging diseases. Certainly all the veterinarians that I talk to are eager for rational solutions that maintain the overall health of our food supply without compromising animal welfare.

Acknowledgements:

Special thanks to Drs. Daniel Hurnik, Andrea Bourque, Shannon Martinson and Enrique Aburto for supplying much of the case material for this report.

Reference:

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Clonality Testing in Dogs and Cats: Polymerase Chain Reaction (PCR) for Antigen Receptor Rearrangement (PARR)

By Noel Clancey, Veterinary Clinical Pathologist

Cytologic diagnosis of canine lymphoid malignancies is often straightforward. However, there are situations that present diagnostic challenges. These include evaluation of fluid or tissue aspirates that contain low numbers of atypical lymphocytes or high numbers of mature-appearing lymphocytes as well as lymph node aspirates in which early stages of lymphoma appear cytologically similar to lymphoid hyperplasia. Evaluation of patients with chronic lymphocytosis is also challenging. In such cases, it would be useful to know whether the lymphoid population is polyclonal (reactive) or clonal (most often neoplastic). To determine this, clonality testing needs to be performed. One such test is polymerase chain reaction (PCR) for antigen receptor rearrangement (PARR).

To understand the basis of this test, remember that B lymphocytes have immunoglobulin (Ig) on their surface and T lymphocytes have T cell receptors (TCR) on their surface. In a situation of benign antigenic stimulation due to infectious or immune-mediated disease, the reactive lymphoid population is composed of cells with variety in the molecular structure of the Ig or TCR. Conversely, a neoplastic lymphoid population originally derived from one cell will consist of many cells expressing the exact same Ig or TCR gene. When this is seen (called clonal expansion or clonality), it is most likely that these cells are neoplastic rather than reactive.

The first step of PARR testing is to isolate DNA from cells obtained via whole blood or aspirate collection. This is performed by using PCR primers directed at regions of the Ig or TCR genes. The amplified PCR products are then loaded onto a polyacrylamide gel and the products separated by electrophoresis. Multiple bands that appear as a ladder on the gel represent different populations of lymphocytes and support benign reactivity. A dominant, single band (one big rung of a ladder) reflects a population of lymphocytes that share an identical DNA sequence and indicates clonality.

At first glance, PARR seems like the perfect test to distinguish reactive lymphoid populations from neoplastic ones. However, there are some limitations. While testing laboratories generate their own sensitivity and specificity values, sensitivity for diagnosing lymphoid neoplasia is generally ~ 65-80% (lower in cats than in dogs) and specificity is generally ~90-95%. Therefore, false negatives occur more often than false positives. False negatives may occur due to several situations, including a malignancy involving a gene to which the primers do not bind or a natural killer cell malignancy in which the tumor cells do not contain a rearranged antigen receptor gene. Rare cases of false positive results in which there is a clonal population of non-neoplastic lymphocytes have included situations of Rocky Mountain Spotted Fever, Lyme disease or infections with *Bartonella* spp. or *Ehrlichia canis.*

The AVC Diagnostic Services Laboratory is now offering the PARR test; the samples are sent to Colorado and turnaround is rapid. If one is knowledgeable about the advantages and disadvantages of PARR testing, it can be a useful test in certain situations. Clinically, it is most commonly used to aid in distinguishing reactive from neoplastic lymphocytes when routine first-line diagnostic procedures such as cytology and histology provide ambiguous results. If you are interested in PARR testing, please feel free to call us at 902-566-0863 for further information.

In response to your requests, we are pleased to announce that Diagnostic Services has added phosphorus to the Canine and Feline Senior Wellness panels at no additional charge.

Infectious Laryngotracheitis in Chickens: A Brief Update

By Carmencita V. Yason, Veterinary Diagnostic Virologist

Infectious Laryngotracheitis (ILT) is a highly contagious avian respiratory tract infection caused by a *herpesvirus* taxonomically classified as Gallid *herpesvirus* 1. It is mainly a disease of chickens but the Infectious Laryngotracheitis virus can also infect pheasants and peafowl.

The disease was first described in 1925 and during the last 15 years, severe ILT outbreaks have been occurring in intensive poultry producing areas in North America and other countries. The morbidity can be very high and the mortality can be from 10% to 70%; recent mortality levels have been 12-16% in layer operations and up to 50% in broiler flocks. ILT is considered to be a disease of serious concern to the poultry industry.

In Atlantic Canada, ILT outbreaks occur sporadically (1-2 per year) in backyard, hobby and fancy flocks of chickens. It is currently a named or reportable disease in all 4 provinces of Atlantic Canada, is an immediately notifiable disease in Canada (under regulations related to the federal *Health of Animals Act*), and is on the list of infectious agents of concern to importing countries such as China and Russia.

Infection with ILT virus results from exposure of susceptible birds to natural field virus or vaccine strains. Infection can result in latently infected birds which become carriers and can transmit the virus to other susceptible birds; the viral shedding usually associated with periods of stress. The virus is transmitted via the upper respiratory system and is shed through ocular, respiratory and oral secretions and aerosols.

Clinical manifestations of ILT can vary from extremely severe, such as gasping for breath and expectoration of bloody mucus, to very mild signs difficult to distinguish from other respiratory diseases. Significant postmortem findings are mainly confined to the respiratory tract and consist of exudative to-hemorrhagic tracheitis, characterized by blood clots in peracute cases. Diphtheritic caseous necrotic plaques are found in the trachea and larynx in subacute and chronic cases while in mild cases, gross lesions consisting only of conjunctivitis and tracheitis are seen.

The distinctive Cowdry-type inclusion bodies, which can be detected by histopathology, are considered to be diagnostic but mainly occur only during the early phase of infection (during the first 3-5 days). Polymerase chain reaction (PCR) testing is more useful and has been shown to be more sensitive than virus isolation in samples that contain other viruses. PCR and virus isolation have similar diagnostic sensitivity during the middle to the end of the infection phase, but PCR is more sensitive than virus isolation in the recovery phase so PCR is the test that is mainly used for confirmation of ILT at the Regional Diagnostic Virology Services.[1] ILT can be also be confirmed by virus isolation in primary cell culture. However, this test is expensive and has a long turnaround time (28-35 days).

The chicken embryo origin (CEO) ILT modified live vaccine has been proven to be effective but has also shown to revert back to virulence, whereas the tissue culture origin (TCO) modified live vaccine does not revert back to virulence. Both CEO and TCO vaccines can infect susceptible birds which can become latently infected and become a source of infection to susceptible birds that have not been vaccinated. A new generation of vaccines, using recombinant technology, is being evaluated and is expected to provide protective immunity with no transmission of clinical disease or production of latent infection in vaccinated flocks.

The eradication of ILT virus from intensive poultry production is definitely feasible as a result of the stability of ILT virus genome, high degree of host specificity and instability of the virus outside its host. The chicken is believed to be the primary host and other gamebird or wildlife hosts are of minor importance. Backyard, hobby and fancy chicken flocks, which are more likely to become a reservoir of the virus, should be considered in the eradication strategy. It has to be stressed that the eradication will be facilitated if commercial vaccines induce protective immunity with no ILT virus transmissibility or development of latently infected birds.

Ongoing surveillance for ILT virus and a rapid and accurate diagnosis are essential for detecting and controlling ILT outbreaks in Atlantic Canada, so appropriate eradication, quarantine, cleaning and enhanced biosecurity measures can be implemented as soon as possible. Please immediately contact your veterinarian, and your provincial laboratory and/or provincial regulatory personnel in your area if ILT or any other respiratory disease is suspected in a chicken flock.

Reference:

1. Alexander HS and Nagy E. Polymerase chain reaction to detect laryngotracheitis virus in the conjunctival swabs from experimentally infected chickens. *Avian Dis*. 1997;41:646-653.

Mysterious Neutrophil Inclusions in a Cat

By Elizabeth O'Neil and Shelley Burton, Veterinary Clinical Pathologists

A 16-year-old spayed female domestic shorthaired cat was presented with a history of vomiting. She had been diagnosed with hyperthyroidism 3 months previously, at which time therapy had been initiated with methimazole. The cat had gained weight and had been doing well clinically until the vomiting began.

Blood was submitted for a serum biochemical panel and a complete blood count (CBC) to the Diagnostic Services Laboratory at the Atlantic Veterinary College. A moderate azotemia of pre-renal or renal origin was seen; a concurrent urine specific gravity was not available to differentiate these. Other changes included mild increases in hepatic enzyme activities and a mild hyperlipasemia. This latter finding was most likely due to azotemia, but hepatic, gastrointestinal or pancreatic disease were other considerations.

A mild neutrophilia and a mild left shift indicating inflammation were seen. Approximately 65% of the neutrophils had few (1-3) to many (>8) small round magenta cytoplasmic inclusions (Figure 1). Considerations for these included phagocytized mast cell granules, hemosiderin, nuclear remnants or toxic granules. A much less likely consideration given the age of the cat and normal skeletal structure was an inherited enzyme deficiency such as mucopolysaccharidosis.

A blood smear submitted from the patient three months earlier was re-examined. No neutrophil inclusions were found; this essentially ruled out an inherited enzyme deficiency. On blood smears from the current submission, the inclusions stained positively with toludine blue and new methylene blue, consistent with mast cell granules. Negative Sudan black and Prussian blue staining helped rule out toxic granules and hemosiderin, respectively. It was suspected that the patient had visceral mast cell neoplasia and that release of granules from degranulated tumor cells were being phagocytized by neutrophils.

The cat continued to have episodic vomiting and weight loss despite good control of the hyperthyroidism. Euthanasia was elected 7 months after the neutrophil inclusions were noted. On the day of euthanasia, ~7% of the neutrophils on blood smears contained phagocytized granules with similar features to those seen earlier. Other significant findings were a mild thrombocytopenia and a very mild neutrophilia. Transmission electron microsopic imaging showed structures consistent with mast cell granules in the cytoplasm of several neutrophils (Figure 2). On post-mortem examination, neoplastic mast cells were seen infiltrating the spleen, liver and bone marrow, confirming the earlier suspicion of systemic mastocytosis.

Wright-Giemsa is a routine cytological and hematological stain which consistently stains mast cell granules.[1] Other stains used to detect mast cell granules include new methylene blue, toluidine blue and thionine.[2] Stains such as Diff-Quik fail to stain or poorly stain mast cell granules.[1] Although mast cell granules or mast cells in circulation are associated with mast cell neoplasia in

Figure 1: Blood smear with neutrophil containing inclusions. Wright-Giemsa, x 100 objective.

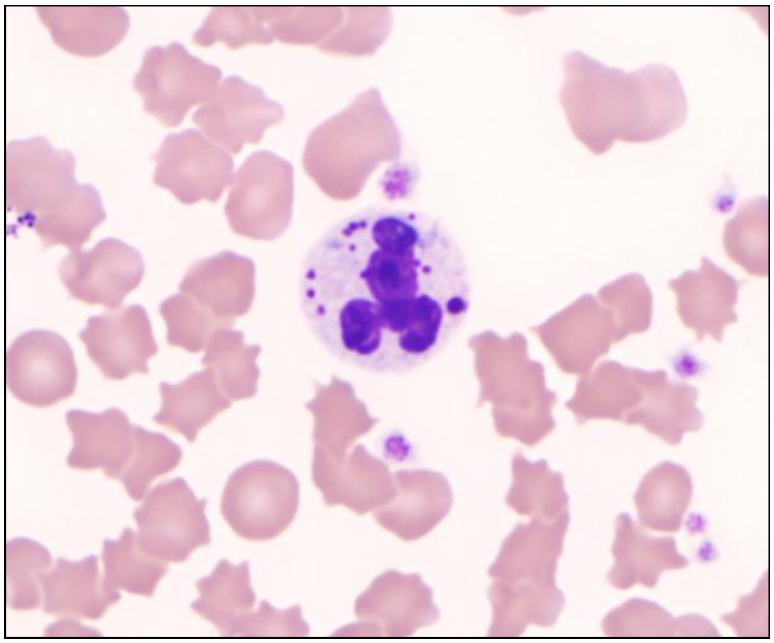
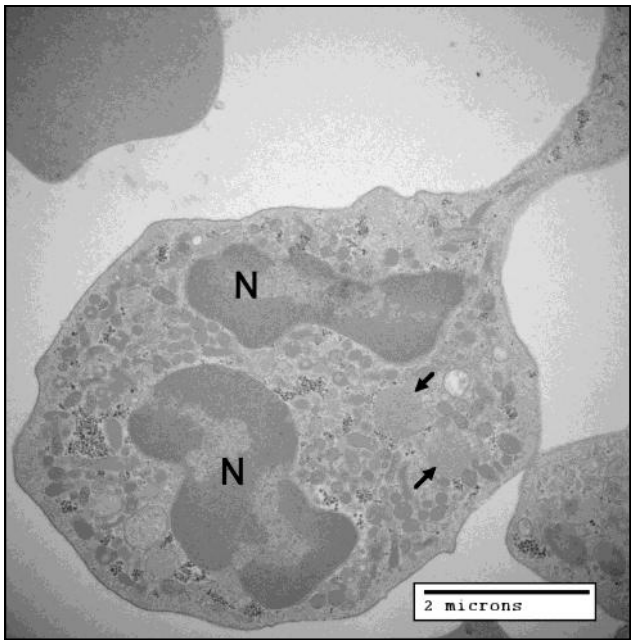


Figure 2: Transmission electron micrograph of a neutrophil with suspect mast cell granules (arrows). Nucleus (N); x 20,000.



the majority of cats, rare circulating mast cells may be seen in other diseases, most notably lymphoma.[3] This is in contrast to the situation in dogs, where circulating mast cells are more commonly associated with inflammation, regenerative anemia or tumors other than mast cell neoplasia.[4]

This case emphasizes the importance of microscopic evaluation of blood smears as well as the value of using different stains. The presence of phagocytized mast cell granules may support a diagnosis of mastocytosis in cats, especially if there is splenic enlargement. In fact, mast cell neoplasia appears to be the most common cause of splenomegaly and splenic disease in cats.[5] Cats with this condition frequently present with vague non-specific signs, including vomiting, anorexia and weight loss.[6] This case also highlights the importance of pursuing further diagnostic work in a patient whose clinical signs have been initially attributed to another disease such as hyperthyroidism. In this case, the blood smear evaluation as part of the CBC raised suspicion for systemic mastocytosis.

Acknoweldgements:

Special thanks to Drs. Alfonso López, Carolyn Legge and Kim Foote for their contribution to this case.

References:

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Laboratory News

By Cornelia Gilroy, Veterinary Clinical Pathologist

Here are some recent happenings in Diagnostic Services:

- Dr. Elizabeth Steeves was here as a locum from September 1st to November 30th as a replacement for Dr. Barbara Horney who was on sabbatical leave. We wish Dr. Steeves all the best as she recently moved back to the United Kingdom.
- Congratulations to previous pathology residents Drs. Carolyn Legge and Soraya Sayi for passing the American College of Veterinary Pathologists (ACVP) certifying examination in anatomic pathology. They are now boarded Diplomates of the ACVP!
- Dr. David Groman presented talks on American Eel Disease surveys and the Veterinary Laboratory Association Quality Assurance Program (VLAQAP), with a booth advertising the VLAQAP at the Conference of the European Association of Fish Pathologists Annual Meeting in September 2011 in Split, Croatia.
- Dr. Gerry Johnson retired from the Department of Pathology and Microbiology in September, 2011. He provided many years of service and teaching in the area of aquatic pathology. We want to wish him all the best!
- Our current pathology residents are Dr. Carlos Mendez Lopez (Anatomic Pathology), Dr. Heather Fenton (Wildlife Anatomic Pathology) and Dr. Dania Villarnovo (Clinical Pathology).
- Dr. Elizabeth (Betsy) O'Neil, who completed her clinical pathology residency in July 2011 presented an interesting mystery case at the American Society for Veterinary Clinical Pathology (ASVCP) annual meeting in Nashville, Tennessee. Only 20 of these cases are selected each year from those submitted. See page 6 of this newsletter for an article on the same case of unusual neutrophil granules.
- Drs. Cornelia Gilroy and Shelley Burton are excited to prepare for a hematology wet laboratory session for the Atlantic Provinces Veterinary Conference (APVC) in April 2012. We hope to see many of you there!

Staff Focus

Dennis Olexson

By Shelley Burton, Veterinary Clinical Pathologist

Dennis Olexson needs no introduction to most Atlantic Canadian veterinarians, as he has been the Manager of the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory since 1986. With his friendly nature, easy accessibility and good humor, he has formed many strong relationships over the years.

Dennis grew up on a grain farm near Rosthern, Saskatchewan, and attended the University of Saskatchewan, where he obtained a BSc, an MSc in Biochemistry and ART (Advanced Registered Technologist) certification from the University Hospital. Dennis was then recruited by Dean Ole Nielsen to operate the Western College of Veterinary Medicine (WCVM) Diagnostic Laboratory, where he served as manager for 10 years. In 1986, Dennis was recruited by Dean Reg Thompson and Dr. Jim Bellamy to establish and manage the new AVC Diagnostic Services Laboratory. He was excited by this opportunity of a lifetime!

Over the past 25 years, Dennis has had a strong impact on both the AVC and the broader veterinary community. He established and developed the Veterinary Laboratory Association Quality Assurance Program (VLAQAP) at the AVC. This is a quality assurance program, run in conjunction with Diagnostic Chemicals Ltd (now Seikisui), which confidentially monitors testing results from veterinary laboratories around the world. Dennis has provided extensive service to the Veterinary Laboratory Professionals organization, which is under the umbrella of the American Society for Veterinary Clinical Pathology (ASVCP). He served as vice-chairperson for 6 years and chairperson for 3 years, a busy role that involved organizing the annual meeting, recruiting speakers and serving on various committees of the ASVCP. Dennis has served in both informal and formal consulting roles for various veterinary laboratories around the world.

Dennis has interests outside the confines of the veterinary laboratory. He enjoys travel, fine dining and doting on his cat, Chester. He is a supremely competent gardener and his yard has even been featured in the magazine, Canadian Gardening!

Dennis is recognized in the AVC Diagnostic Laboratory for many things, including his insistence on quality, his tidiness (to the point where we know never to leave an important scrap of paper unattended!) and his pride in touring visitors through the laboratory. His kindness to newcomers is well known and he particularly takes the pathology residents under his wing, some of whom have moved to PEI from far away. In summary, it would be hard to imagine the AVC Diagnostic Services Laboratory without Dennis Olexson - his hard work has helped to make it one of the world's premier veterinary laboratories today!

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.