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Diagnostic Update

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Welcome to the New Associate Dean of Professional Services

by Cora Gilroy, Veterinary Clinical Pathologist

The Associate Dean of Professional Services is a new position at the Atlantic Veterinary College (AVC), with the responsibilities of overseeing the Veterinary Teaching Hospital, Diagnostic Services and Laboratory Animal Resources. A primary role of this position is to promote and develop professional services and service delivery, as this encompasses one of the three core activities of the AVC, which are teaching, research and service. Dr. Darcy Shaw is the first person to fill this position, effective June 1st, 2009.

A native of Moose Jaw, Saskatchewan, Dr. Shaw graduated with distinction from the Western College of Veterinary Medicine (WCVM) in 1983. Following 2 years in private practice, he returned to WCVM and completed a residency and graduate program in small animal internal medicine. In 1987, he moved to PEI to become one of the first AVC faculty members. The earlier phase of Dr. Shaw's career saw him concentrate on clinical service, teaching and research, with a focus on renal disease. He garnered 6 teaching awards and became a Diplomate of the American College of Veterinary Internal Medicine. Dr. Shaw has a strong interest in client communications and helped initiate a rigorous 4th year communication rotation at AVC.

As his career progressed, Dr. Shaw had the honor

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of serving as president of the Canadian Veterinary Medical Association (CVMA) in 2000. In his desire to build administrative strengths, Dr. Shaw served as Director of the AVC hospital, completed a Master of Business Administration (MBA) and served a 5 year term as Chair of the Department of Companion Animals. His MBA project focused on the challenges and strengths of the AVC admissions process and resulted in substantive changes made to this area. Throughout his career, Dr. Shaw has taken the opportunity to build experience in many aspects of veterinary medicine, such as by serving as the CVMA member on 8 American Veterinary Medical Association accreditation site visits to veterinary colleges around the globe.

Although Dr. Shaw still has family ties to western Canada, PEI has been his home for 22 years. He feels lucky to live in Atlantic Canada and looks forward to increased interaction with Diagnostic Services clients. Dr. Shaw lives in Stratford, PEI, with his wife, Shelley, and their two Siamese cats, Wally and Norm.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

by C. Anne Muckle, Veterinary Bacteriologist and J Trenton McLure, Large Animal Medicine Internist

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasingly important human health issue, not only due to serious infections acquired by patients in hospitals (nosocomial infections), but also due to the recent increase in community-acquired infections.

Strains of MRSA are typically resistant to all beta-lactam antibiotics (such as amoxicillin, oxacillin, methicillin, penicillin and cephalosporins) and may also be resistant to other antibiotics. Methicillin resistance is due to an altered penicillin-binding protein (PBP2a) encoded by a gene called the staphylococcal chromosomal cassette *mec* (SCC*mec*) gene. Five types of SCC have been described: types I, II and III in hospital-acquired MRSA strains (HA-MRSA) and types IV & V in community-acquired MRSA strains (CA-MRSA). In Canada, six epidemic clones of MRSA (CMRSA) have been reported.

Humans can be colonized with MRSA without being infected; infection occurs when MRSA enters the body, usually through skin. Most MRSA cases are skin, surgical site and wound infections, but some are life-threatening infections such as pneumonia or septicemia. HA-MRSA infections are associated with hospitalization, surgery, presence of an invasive medical device such as a catheter, dialysis or residence in a long-term care facility. Even stethoscopes of emergency medical personnel have been found to be harbouring MRSA! CA-MRSA are of particular concern as they occur in healthy people without any predisposing risk factors and produce more serious tissue damage due to production of the Panton-Valentine leukocidin toxin.

MRSA is also an emerging infection in veterinary medicine, with infections reported originally in horses and dogs, but now also in cats, swine and cattle. The reason for this is unclear, but for humans, the use of fluroquinolones may predispose to carriage of MRSA strains.

Similar to humans, animals can be either colonized with MRSA with no apparent illness or have clinical infection. A major issue is the potential for zoonotic transmission of MRSA from farm and companion animals to humans or visa versa. Companion animals are of particular concern because of intimate contact with their owners, with immunocompromised people being particularly at risk. MRSA in farm animals is cited as an international public health issue because intensive farming practices could provide a growing reservoir of this superbug worldwide. Several investigations have confirmed that MRSA can be transmitted

between animals and humans. Significantly, **both** animal-to-human and human-to-animal transmission is occurring. One survey of horses and horse personnel found that the majority of human and equine isolates were the same CMRSA subtype and had the SCC*mec*IV gene. Veterinarians, especially equine practitioners, have a higher rate of MRSA colonization than the general population. At the Atlantic Veterinary College (AVC), Dr. J McClure has been screening all equine patients admitted to the veterinary teaching hospital for MRSA since June of 2007. To date, the carrier rates are <1% among the AVC hospitalized horses which is lower than that reported from horses admitted to the Ontario Veterinary College (2.7%).

Given this zoonotic connection, veterinarians may be asked by clients to screen house pets for MRSA, usually on the advice of physicians who are investigating human MRSA infections in households. Pet owners might be told that their dog or cat is the source of infection and should be destroyed. Proper client education by veterinarians is critically important, since the origin of MRSA in dogs and cats is most likely from colonized or infected humans. Decolonization therapy used in humans is not a routine practice for animals and more research is needed before it can be recommended. Fortunately, MRSA- positive pets can become decolonized when their exposure to infected humans is prevented. One strategy to break the cycle of infection is to temporarily remove the pet for several weeks while the humans are treated. The most important message we can give to clients is that the best way to decrease the chance of infection with MRSA is by practising good infection control, good hygiene and hand washing.

Dogs and cats can be screened for MRSA by collecting both nasal and perineal/anal swabs for specialised MRSA culture. For horses, an intra-nasal swab is sufficient. At the AVC, MRSA screening is conducted by Dr. J McClure through the Diagnostic Services Bacteriology Laboratory.

References:

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Testing for Adrenal Disease in Ferrets

by Shelley Burton, Veterinary Clinical Pathologist

Adrenal gland disease is common in ferrets, with an estimated 70% of pet ferrets in the United States affected during their lifetime. This unique condition is different from hyperadrenocorticism in dogs. In ferrets, adrenal hyperplasia or benign or malignant neoplasia causes excessive secretion of sex hormones as opposed to the excessive cortisol or adrenocorticotropic hormone secretion seen in canine hyperadrenocorticism. Clinical signs vary, with progressive alopecia being most common. Other signs include vulvar swelling in females and stranguria or even complete urethral blockage in males due to prostatic enlargement. Rarely, excessive estrogen secretion results in bone marrow suppression.

Laboratory diagnosis relies on measurement of various sex hormones in serum. Standard current testing involves evaluating concentrations of a panel of three sex hormones (estradiol,

17-hydroxyprogesterone and androstenedione). Panel evaluation is more cost-effective and has higher sensitivity than a stepwise evaluation of individual hormones. If any one of these hormones is appreciably increased in a patient with clinical support, a diagnosis of adrenal disease is made. The current cost of this panel to Atlantic Veterinary College Diagnostic Services clients is $150.00. This testing is done at the University of Tennessee and our laboratory ships samples to this location using a Canada-US border broker.

A ferret hormone panel analysis requires a serum sample with a volume of at least 1.0 ml, ideally collected from a fasted animal to avoid lipemia. To avoid sample hemolysis, the serum is removed after sample clotting and centrifugation, and placed in a new red-topped tube. The sample is refrigerated and kept cool using gel packs during shipment. It is ideal for our laboratory to receive the sample early in the week so that it can be brokered across the border without concern for weekend delays.

Canine Oral Melanomas - Still Dangerous?

By Shannon Martinson, Veterinary Anatomic Pathologist

Melanomas are one of the most common oral tumours of dogs. The histological appearance of these tumours varies greatly, from masses composed of heavily pigmented melanocytes of uniform shape and size, to those composed of highly anaplastic cells with or without pigment. Criteria used to differentiate between benign (melanocytoma) and malignant (melanoma) melanocytic neoplasms are not sharply defined in dogs, and they are one of the few tumours in which location has been commonly used as a prognostic indicator. It has long been thought that all canine melanocytic tumours in the oral cavity and lips, regardless of their histological appearance, should be considered malignant, while those in the skin are more often benign.

Oral melanomas most commonly arise in the gingiva and lips, with fewer cases arising in the lingual, buccal, pharyngeal, tonsillar and palantine epithelium. Presenting clinical signs include dysphagia, halitosis, ptyalism, bleeding and occasionally fracture of the mandible. They tend to exhibit rapid growth, aggressive local invasion and recurrence following excision. It has been reported that 70 – 90% of these tumours metastasize independent of their location in the oral cavity; common sites of metastasis include the regional lymph nodes, the lung and the viscera. The median survival for untreated dogs can be as short as 65 days, while survival periods following treatment range from 3 to 8 months.

Although many oral melanomas do exhibit aggressive behavior with high rates of recurrence following removal and early metastasis, there is now evidence that these tumours may not always carry such a grave prognosis. It has recently been shown that dogs with less aggressive subgroups of oral melanomas may survive for long periods following surgery, with or without adjuvant therapy. In a recent study[1], post-surgical information was obtained for 64 dogs with histologically well-differentiated melanoma of the oral cavity or lips. These dogs were treated with local excision of the mass with no adjunct therapy. Sixty-one of the 64 dogs were either alive at the end of the study (34) or died of unrelated causes (27), with a median survival of 3 years after surgery. Only 3 of the 64 dogs died of tumour-related causes and only 2 additional dogs had local recurrence of the mass; these 5 dogs all had masses within the oral cavity.

While some reports state that tumour size and location (lips versus oral cavity) of oral melanoma in dogs are unrelated to prognosis, others have reported that tumours arising within the oral cavity are more aggressive than those arising in the lips. It seems likely that lip

lesions are recognized earlier and perhaps are more easily removed than those within the oral cavity. The same may be said about tumour size, with smaller lesions being easier to completely excise.

Because invasive growth and early metastasis are still commonly reported with canine oral melanomas, it remains prudent to excise all melanocytic lesions in the oral cavity and on the lips. While these tumours will likely continue to be regarded with suspicion by both clinicians and pathologists, there remains hope for prolonged survival of dogs with well-differentiated melanocytic tumours after surgical excision. This may be especially true for dogs with small masses and for those with tumours occurring on the lips where complete surgical excision is more likely.

Reference:

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Atypical Cells on a Blood Smear: What Do They Mean?

By Cora Gilroy and Shelley Burton, Veterinary Clinical Pathologists

Have you ever had atypical cells identified on a blood smear from one of your patients? What are these cells and what does their presence mean?

Atypical cells (Figure 1) are large immature cells of any cell lineage. When hematology technologists at the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory identify atypical cells, they are numerically placed in a general immature category of the white blood cell (WBC) differential count and then a notation on the report identifies them as atypical cells. This is necessary because the immature category on the differential is also used for other cell types, such as mast cells or myelocytes, which do not fit into regular differential count categories.

There are two general possibilities for the presence of atypical cells. First, these cells could merely be benign reactive lymphocytes if the animal is experiencing antigenic stimulation from an infectious or immune-mediated disease or a recent vaccination. While most benign reactive lymphocytes have a classic morphology, some become more unusual appearing and fit an atypical morphology. The other possibility is that the atypical cells are associated with an acute leukemia or myelodysplastic syndrome.

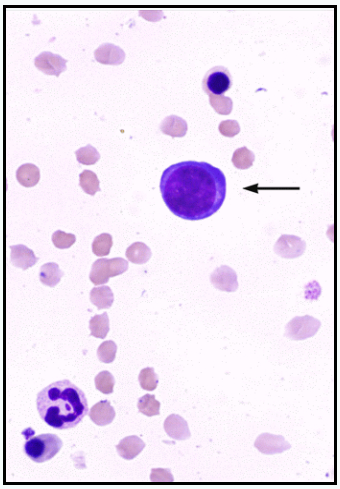

Figure 1: Atypical cell (arrow), a neutrophil and two nucleated RBCs in a feline blood smear.

To decide if antigenic stimulation, an acute leukemia or myelodysplastic syndrome is occurring, the historical, clinical and other diagnostic findings are evaluated. Does the animal have support for immune-mediated or infectious disease? Are there cytopenias or enlarged organs which support an acute leukemia? Are there cytopenias and hemic cell maturation abnormalities to suggest a myelodysplastic syndrome? In some cases, very high numbers of circulating atypical cells allow a diagnosis of an acute leukemia from the

complete blood count (CBC) alone. For example, in a dog with a total WBC count of 100 x 10^9/L in which 90% of the cells are atypical, an acute leukemia diagnosis is made without further testing. If there are low numbers of atypical cells present, the situation is more diagnostically challenging. If there is strong clinical support for an inflammatory condition, often the CBC is monitored for the continued presence or disappearance of these cells as the underlying condition is treated. However, if there is no strong support for an inflammatory condition or if the atypical cells persist, further diagnostic testing, particularly bone marrow evaluation, is warranted.

When atypical cells are seen, cytochemical staining can help identify possible cell lineages. This is important if an acute leukemia is present, as it can help guide chemotherapy. A cytochemical stain commonly used at our laboratory is Sudan Black B. This stain binds to intracellular lipids and some non-lipid cellular components. Moderate to strong staining occurs in granulocytes and weak staining in monocytes. The following table outlines expected results for Sudan Black B staining in various cell lines:

Negative	Positive
Lymphocytes	Neutrophils
Erythroid cell line	Eosinophils (except cats)
Early undifferentiated cells	Monocytes
Megakaryocytes	Basophils – sheep & cattle
Basophils – dog, cat & horse	

If an acute leukemia is present and the atypical cells are Sudan Black B negative, a lymphoid leukemia is most likely, **but** it is important to realize that this cannot be specifically diagnosed, as other lineages have cells that are also negative with this stain. If the cells are Sudan Black B positive, we know we are dealing with a monocytic or granulocytic leukemia and the chemotherapeutic regime chosen will be different than if we suspect a lymphoid leukemia. To definitively identify the lineage of the atypical cells, further testing such as immunocytochemistry or clonality testing at referral laboratories is required.

If atypical cells are noted on an in-clinic blood smear and further evaluation is desired, it is recommended to submit to AVC Diagnostic Services both 1-2 air-dried unstained blood smears and an EDTA whole blood sample (lavender top tube) for a full CBC rather than smear evaluation alone. This is much less expensive and allows both a fuller interpretation by the duty clinical pathologist as well as the application of cytochemical stains if needed. Please feel free to call our laboratory if you have any questions concerning this topic – we would be happy to help!

References:

1. Allison R, Meinkoth J. Hematology without the numbers: in-clinic blood film evaluation. *Vet Clin Small Anim*. 2007;37:245-266.

2. Beutler E, Lichtman MA, Coller BS, Kipps TJ. 1995. Williams Hematology, 5th Ed. New York: McGraw-Hill, Inc.

3. Feldman BF, Zinkl JG, Jain NC. 2000. Schalm's Veterinary Hematology, 5th Ed. Philadelphia: Lipponcott Williams & Wilkins. pp 3-11.

Laboratory News

What's New in Diagnostic Services

by Shelley Burton, Veterinary Clinical Pathologist

- Seven individuals from Diagnostic Services (Andrea Bourque, Melanie Buote, Shelley Burton, Cora Gilroy, Barbara Horney, Dennis Olexson and

- Sandra McConkey) traveled to Halifax in April to provide lectures and wet laboratories at the Atlantic Provinces Veterinary Conference. It was a resounding success and we look forward to future participation with this fantastic conference!

- Dr. Darcy Shaw moved into the new position of Associate Dean of Professional Services on June 1st, 2009. A full article on Dr. Shaw is found on page 1.

- Dr. Gerry Johnson finished his term as department chair in Pathology & Microbology and Dr. Fred Kibenge took over this role on June 1, 2009. We wish Dr. Johnson well as he pursues his many interests in aquatic pathology.

- Darren MacEachern received a permanent appointment as a post-mortem technician following the resignation of Jim Carlsen, who is now working at the University of Calgary Faculty of Veterinary Medicine.

- On June 7-10th, Charlottetown was the site of the annual meetings of the Canadian Animal Health Laboratorians Network (CAHLN) and the Canadian Association of Veterinary Pathologists. The CAHLN conference had over 135 participants, including many from Diagnostic Services. One highlight was the awarding of Laboratorian of the Year to Dr. Gerry Johnson. The local organizing committee, chaired by Dr. Carmencita Yason, deserves congratulations for their hard work in preparing for this exciting event.

- Our resident amateur photographer, Dr. Noel Clancey, received the Best in Show award out of 229 entries at the PEI Photography Club annual show in May. Dr. Clancey has been an asset to the clinical pathology group, as he can utilize his photography skills to obtain great images of cytology and blood smears!

Staff Focus

Robyn MacPhee

By Linda Ruschkowski, Veterinary Laboratory Technologist

Robyn MacPhee, one of the newer members of our Diagnostic Services team, grew up in Stratford, PEI, and attended the University of Prince Edward Island (UPEI) where she received her Bachelor of Science degree. Following graduation, she worked at UPEI in the field of nutritional science research. In October of 2007, she joined the Diagnostic Services virology laboratory as a technologist.

Robyn is a world class athlete in the sport of curling. Many will recognize her from television this past year when she skipped for the PEI team at the Scotties Tournament of Hearts. She began curling at the tender age of 5, where she was the youngest member to ever join the Charlottetown Curling Club "Little Rocks" program. She went on to play at the Canada Games in 1999, the National Junior Curling Championships from 2000-2002 (where she won a silver and 2 gold medals), the World Junior Championships in 2001 and 2002 (where she won a gold and a bronze medal), and the Scotties Tournament of Hearts in 2003, 2005 and 2007-2009.

In the winter, Robyn spends all her free time training for curling and enjoying the pleasures of this game, something her whole family loves. In the summer, she enjoys relaxing at her cottage in Wood Islands. Whether it's the pressure of the game or the busy virology laboratory workload, Robyn stays as cool as a cucumber. We are all very proud of Robyn's athletic accomplishments and look forward to cheering her on again during next year's curling season!

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.