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# Diagnostic Update

## Diagnostic Services Moves Toward ISO Accreditation

*By Shelley Burton, Veterinary Clinical Pathologist*

In the fall of 2009, the Atlantic Veterinary College (AVC) welcomed Ms. Karen Smith as project leader to prepare the Diagnostic Services laboratories for testing accreditation by the Standards Council of Canada. A native of Peakes, Prince Edward Island (PEI), Karen received a Bachelor of Science degree and completed graduate courses and research toward a Master of Science degree at the University of PEI. Beyond her academic training, Karen brings the benefit of extensive prior experience in the highly regulated animal health industry to her current role.



While working for a leading global pharmaceutical company, she contributed technical expertise for several years to the research, development, manufacture, testing and control of animal vaccines. During the past ten years, she managed the quality control, quality assurance, regulatory compliance and licensing programs for the company. These influential positions

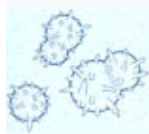
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required meticulous attention to detail as well as excellent organizational, written and verbal communication skills.

In her new position with the AVC, Karen's initial goals are to develop and implement a Quality Management System for Diagnostic Services to form a regulatory umbrella for operational policies, and to achieve ISO 17025 accreditation for both the Toxicology and Analytical Services and the Regional Diagnostic Virology Services laboratories. Testing laboratories accredited to ISO 17025



standards must meet rigorous technical and record keeping criteria that assure the competence of the laboratories and the accuracy and reliability of test results, bringing to their clients internationally recognized high quality standardized services. Obtaining this accreditation at the AVC will not only acknowledge the already excellent work performed, but will ensure that diagnostic tests and results conform to specific expectations and requirements to greater enhance customer confidence.

Once these two laboratories at the AVC are accredited, Karen hopes to assist with ongoing quality assurance and to prepare other sections of Diagnostic Services for standardization, making it one of the few ISO 17025 accredited veterinary laboratories in North America. While maintaining high client satisfaction is the primary objective of this endeavour, boosting personnel pride through recognition of excellent work performed and facilitating testing traceability are some of the other anticipated benefits.

Karen is excited by the challenges of this project, and finds the exposure to new research and management ideas very refreshing and interesting. As she enjoys promoting continuous learning and improvement, Karen is looking forward to working with AVC colleagues to also establish a formal program for training personnel in ongoing quality and ISO 17025 compliance management. Diagnostic Services is very pleased to have a person with Karen's strengths and experience leading this exciting process.

## **The Dangers of Gambling: An Unusual Source of Zinc Toxicosis**

*By Noel Clancey, Veterinary Clinical Pathologist*

Clinicians at the Atlantic Veterinary College were recently presented with a 16 month old spayed female mixed breed dog with a one week history of anorexia, lethargy, diarrhea, vomiting and difficulty rising. The dog had been

tentatively diagnosed with immune-mediated hemolytic anemia (IMHA) but was not responding to appropriate therapy. Hematologic evaluation revealed a marked macrocytic hypochromic anemia which was markedly regenerative. No spherocytes or Heinz bodies were observed. Abdominal radiographs revealed an intestinal metallic foreign object. The serum lead concentration was within reference limits while the zinc concentration was markedly increased at 24.4 ppm (toxic reference interval = 10 – 32 ppm). The foreign object promptly progressed to the rectum and was manually removed. It consisted of a



*Partially eroded gambling die (on the left) removed from a dog*

partially eroded gambling die with exposed metal surfaces. Fortunately, this dog made a full recovery and is doing well.

Zinc is an essential element required for proper growth and development and is present in approximately 200 metalloenzymes. Zinc can be found in many household items, including calamine lotion, zinc oxide ointment, shampoos, paints, galvanized products used in fencing, coating for nails and in metal pet kennels as well as the nuts and bolts from transport kennels. One cent coins from the United States minted after 1982 are made predominantly of zinc with a copper coating. In Canada, pennies minted between 1997 and 1999 are composed of 98.4% zinc, while those produced before and after this period have almost no zinc. Once ingested, erosion by the stomach acid can release zinc from these objects, leading to toxicosis.

Acute zinc toxicosis has been reported in dogs but not cats, likely due to the more discriminatory eating habits of cats. Once ingested, zinc can result in hemolytic anemia,

pancreatitis, gastrointestinal dysfunction and acute renal failure. The most common clinical signs include vomiting, lethargy, anorexia, diarrhea and pigmenturia. The most consistent clinicopathologic findings include hyperbilirubinemia and a moderate to severe anemia that is often macrocytic, hypochromic and regenerative. The anemia is hemolytic in origin, but the pathophysiology of zinc-induced hemolysis is not fully known. Inhibition of glutathione reductase and enzymes of the hexose-monophosphate-shunt pathway have been primarily suspected. Lack of function of these enzymes makes erythrocytes more susceptible to oxidative damage. Oxidative damage typically results in the production of Heinz bodies, but the presence of Heinz bodies associated with zinc toxicosis has been inconsistently reported. A recent review of zinc toxicosis in dogs reported Heinz bodies to be present in only 33% of 19 patients<sup>1</sup>. While oxidative damage may be partly responsible for the hemolysis, it may not be the only mechanism involved.

Spherocytes have also been reported in some cases of zinc toxicosis, making them very difficult to differentiate from an IMHA. Similar to Heinz bodies, spherocytes are inconsistently described and reported to be present in only approximately 20% of affected dogs<sup>1</sup>. When present, the degree of spherocytosis is typically mild. Patients with marked spherocytosis, as well as agglutination, should be more strongly suspected instead to have IMHA.

Other clinicopathological abnormalities can include increased aspartate aminotransferase (AST) activity, possibly as a result of muscle hypoxia or erythrocyte hemolysis. Other findings can include elevated serum urea concentration secondary to gastrointestinal hemorrhage and hyperamylasemia and hyperlipasemia due to pancreatic inflammation. Prolongation of the partial thromboplastin time (PTT) has also been reported in some dogs with zinc-induced hemolysis, possibly due to inhibition of intrinsic pathway coagulation factors. The prothrombin time (PT) is generally within reference limits.

In conjunction with the above findings, diagnosis of zinc toxicosis can be suspected by seeing metallic dense foreign objects on radiographs. These are often present in the stomach or intestines but may not be present if the patient has vomited the foreign body or passed it in the feces. Topical zinc sources such as zinc oxide ointment are not radiographically detectable, reinforcing the importance of obtaining a thorough history. Definitive diagnosis requires finding elevated serum zinc levels. The reference interval for zinc in dogs is 0.7 to 2.0 ppm. Most dogs with confirmed zinc toxicosis have zinc concentrations > 10 ppm. Blood should be collected into specific tubes (typically with a royal blue rubber stopper) for trace element measurement. Lavender-topped tubes must be avoided as these can contribute up to 5.6 ppm of zinc to the sample. Serum red-topped tubes should also be avoided.

Removal of any ingested source of metal by gastroendoscopy, gastrotomy or enterotomy is critical to successful treatment. Blood transfusion is often required in severely anemic patients, as is close monitoring of coagulation status to detect disseminated intravascular coagulation. Symptomatic and supportive care to correct and maintain hydration, electrolyte balance and acid-base status is important. As acute renal failure is a potential sequela of zinc toxicosis, adequate renal perfusion is essential. Gastrointestinal tract protectants, antiemetics and antimicrobials are commonly instituted. Aminoglycosides should be avoided due to their nephrotoxic potential. Chelation of zinc with calcium disodium EDTA has been proposed. However, its use is controversial since chelation treatment may actually increase intestinal absorption of zinc that has already entered the gastrointestinal tract. Daily monitoring of the complete blood count, serum chemistry, urinalysis and serum zinc concentrations will help assess the success of treatment.

Prognosis for recovery is fair to good with early detection and treatment. However, late detection and/or the development of sequela such as pancreatitis, acute renal failure, disseminated intravascular coagulation or

hepatic necrosis can prolong hospitalization and even result in death. Prior to seeing this case, the clinical pathologists at the AVC were unaware that gambling dice could contain zinc. The hematologic findings were not strongly suggestive of zinc toxicosis as there were no Heinz bodies or spherocytes present. It was the presence of a metallic foreign body and the high serum zinc concentration which allowed the diagnosis of zinc toxicosis to be made in this case.

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## A Client, a Kid and a Frog walk into your Clinic...

By María Forzán, Veterinary Anatomic Pathologist

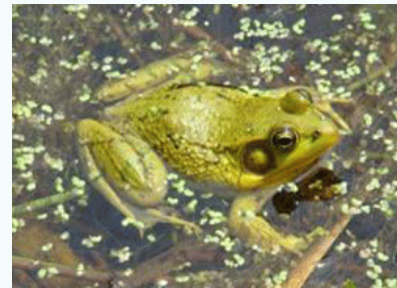
Frogs and salamanders imported from Asia or Latin America have become somewhat popular pets. Most people who are truly fascinated with amphibians have a vast knowledge on the husbandry and even medical aspects of their animals. The amateur frog enthusiast or the child who sees a frog in a pet shop and simply must have it, however, may show up at your clinic to ask for advice. Don't panic! Below are a few short answers to the most common questions about husbandry and infectious diseases in captive amphibians.

### What do I feed my frog or salamander?

Obviously, this depends on the species. To identify native frogs, try the online site for FrogWatch<sup>1</sup>. Books and websites are available to provide adequate husbandry and diet information, including Amphibian Medicine and

Husbandry<sup>2</sup> and the free online Association of Zoos and Aquariums Amphibian Husbandry Guide<sup>3</sup>.

### Can you give my frog a physical?



Yes, you can! Although you may want to consult an exotics veterinarian for complicated cases and

procedures such as blood collection, regular physical examination is fairly simple. Before you start, ask about other frogs in the collection and check if husbandry and feeding are appropriate to the species. Now, to examine a frog you'll need non-powdered gloves (not latex) moistened with water. Look at the posture of the frog and then check for blinking reflexes (touch the eyes gently), righting reflexes (put the frog on its back and watch it right itself) and withdrawal reflexes (extend the limbs distally and watch them contract). Loss of any of these is indicative of a serious illness.

### I brought a new frog or salamander into my tank. A week later the others started dying. What do I do?

If there are no habitat or diet changes, you need to consider the two main infectious diseases that cause high mortality in amphibians: chytridiomycosis (caused by *Batrachochytrium dendrobatidis*) and ranavirus infection. Chytridiomycosis will mainly kill adult and recently metamorphosed frogs, but will not kill tadpoles or salamanders. Ranavirus tends to kill tadpoles of both frogs and salamanders. It may also kill salamanders of any age and adult frogs suffering from concurrent disease. Submission of one of the dead animals to a diagnostic laboratory assists in diagnosis. Fix the dead amphibian in 70% ethanol (prepared with distilled water, not tap water), and send it to the diagnostic laboratory. Ethanol allows both histology and PCR tests to be run on the tissues.



### **The diagnosis came back as chytridiomycosis. How do I treat it?**

Itraconazole at 0.01% solution in 0.06% saline in a 5-minute bath for 11 days<sup>4</sup> can be used. Fortunately, frogs have a ventral patch that absorbs water and solutes, so all you have to make sure is that the frog sits in the solution for the full time period.

### **My frog has small lumps on its fingers and other parts of the body. What is it?**

Mycobacterial and other infections can cause granulomas in the digits of frogs. Definitive diagnosis requires a histologic examination of the lesions, either from a biopsy or necropsy. If awaiting biopsy results, the owner should be advised to use non-powdered gloves when handling the frog as the infection can be zoonotic. Unfortunately, if a frog has mycobacteriosis, euthanasia is recommended, mainly because of the risk of zoonotic transmission.

### **How can you euthanize a frog or salamander?**

One of the easiest and kindest ways is to immerse the amphibian in ethanol, starting with a 5% solution for sedation until it becomes unresponsive, and then submerging in a 20-70% solution to euthanize it<sup>2</sup>. Using ethanol is not only humane but allows histology and PCR testing if the body is to be sent to a laboratory for necropsy.

### **Does the AVC Diagnostic Services Laboratory accept submission of amphibian cases?**

Yes! Our anatomic pathologists, particularly Dr. María Forzán, have an interest in amphibian pathology and would be pleased to help you by evaluating biopsies or performing necropsies on frogs or salamanders submitted.

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2. K. M. Wright and B. R. Whitaker. *Amphibian Medicine and Husbandry*. Krieger Publishing Co., 2001
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## **Testing for Ovarian Remnants in Cats**

*By Rob Lofstedt, Veterinary Theriogenologist*

It is not uncommon for a cat owner to return to a veterinary clinic complaining that their previously spayed cat is now back in heat. This can happen months or even years after the surgery, especially in the spring or early summer when the increase in day length stimulates folliculogenesis.

The reason for the return to heat is due to the fact that the feline ovary has no protective bursa (as seen in bitches). As a result, ovarian tissue is sometimes fractured during ovarian removal, even with fairly careful surgical technique. These fragments of ovarian tissue implant on the omentum or peritoneal surface. Occasionally, ovarian remnants are found in the area of the uterine bifurcation in cats. This may be due to involuntary transplantation of ovarian tissue after handling the ovaries. After transplantation, the fragment or fragments of ovarian tissue grow. They are responsive to gonadotropins and when the breeding season begins, they produce follicles and induce behavioural estrus.

What should practitioners do if faced with a

situation of possible ovarian remnant syndrome? The first step is to verify that the cat is indeed in estrus using vaginal cytology.

If estrus is supported by a high superficial cell index on vaginal cytology, one should administer 25 micrograms of GnRH intramuscularly. Most mixed practices stock GnRH. After the injection, one needs to wait about 10 days before obtaining a blood sample to assess a serum progesterone concentration. If it is greater than 2 ng/ml, it indicates that ovulation has occurred and also substantiates the assumption that the cat does indeed have functional ovarian tissue. A laparotomy is then required to remove the remnant. The remnant can be located anywhere but is most often seen on the peritoneal surface just caudal to the kidneys. Sometimes, ovarian remnants are found on the kidney capsule itself.

The luteal tissue of a cat is pale yellow-to-pink in color and is easier to identify during surgery than the small bubble-like follicles that represent an active ovarian fragment. This is why it is preferable to induce ovulation and to look for luteal tissue than it is to look for ovarian fragments without corpora lutea. Any material retrieved should be submitted for histological evaluation to determine if it is truly ovarian tissue.



If the ovary cannot be located or the owner refuses the surgical option, ovulation can be induced using GnRH to provide

a six week estrus-free period. This treatment may be required two to three times each year, until the cat returns to seasonal anestrus. The long-term effects of the treatment have not been studied. If you are considering this treatment, please contact the Atlantic Veterinary College Theriogenology Service for more information.

To prevent ovarian remnant syndrome in cats, the ovaries must be removed with great care to

avoid tissue fracture. As well, the instrument used for ovarian removal should be discarded after ovariectomy and not be used to retrieve the uterine bifurcation for hysterectomy.

## A Routine Case of Feline Eosinophilic Granuloma...or is it?

*By Andrea Bourque, Veterinary Anatomic Pathologist*

Pathologists at the Atlantic Veterinary College Diagnostic Services Laboratory recently saw a fascinating case. A 12 year-old male castrated domestic shorthaired cat presented with a ~6 cm diameter oval area of ulceration on the flank and two smaller thickened and ulcerated skin lesions on the ventral chest. Biopsies were submitted for histopathologic examination. Both clinically and microscopically, features were suggestive of feline eosinophilic granuloma complex.

However, in a few areas where the epidermis was intact, there were microscopic indications of a viral infection and intranuclear inclusion bodies were present. Immunohistochemistry confirmed our suspicion of feline herpesvirus infection.

Ulcerative dermatitis due to feline herpesvirus infection is uncommon. Lesions most commonly occur on the face but may occur elsewhere. Affected cats may or may not have a history of upper respiratory disease and may exhibit sneezing and excessive tearing. Stress, concurrent disease and immunosuppression due to environmental stressors and treatment with glucocorticoids have been implicated as triggers for herpesvirus infection. Because of the prominent eosinophilic component to the inflammation present in these lesions, this disease has been misdiagnosed as allergic dermatitis or lesions of feline eosinophilic granuloma complex. However, unlike these latter conditions, lesions in cats with feline herpesvirus ulcerative dermatitis worsen following treatment with glucocorticoids. Thus, response to therapy can be used as a diagnostic tool, especially in cases where the overlying epidermis is largely lost. In this case, the cat was doing well and the biopsy sites were

healing well two weeks following the surgery. It is not known if this cat had been treated with glucocorticoids and unfortunately, he was later lost to follow-up.

This case illustrates the importance of not assuming that all situations of eosinophilic dermatitis in cats are due to allergic disease or eosinophilic granuloma complex. Careful clinical and pathologic evaluation is needed to identify feline herpesvirus infection in these cases.

## Laboratory News

### What's New in Diagnostic Services

*by Shelley Burton, Veterinary Clinical Pathologist*

Diagnostic Services continues to be a fun and busy place to work! Here are some recent happenings:

- Of the 20 interesting mystery cases chosen by the American Society for Veterinary Clinical Pathology (ASVCP) to review at their annual meeting, two of them were from the AVC! One concerned an anemic foal presented by our clinical pathology resident, Dr. Betsy O'Neil. The other case was a fox with babesiosis submitted by Dr. Noel Clancey and presented by Dr. Shelley Burton.
- To maintain client service if illnesses occurred, we engaged in continuity planning for the possibility of personnel absences due to H1N1 infection.
- Dr. Alfonso Lopez, one of our anatomic pathologists, was honored to receive a lifetime achievement award in pathology by the Mexican Association of Veterinary Pathologists during their annual meeting held in September in Puerto Vallarta. Dr. Lopez has made a tremendous

contribution to pathology at the AVC. He has a particular interest in respiratory pathology and is the author of the chapter on this system in the prestigious textbook, McGavin and Zachary's Pathologic Basis of Veterinary Disease.

- With the help of Ms. Karen Smith, we are working to improve our quality assurance status by striving for ISO accreditation. Please see the full article on page 1.
- We welcomed back Dr. Darren Wood from the Ontario Veterinary College as a locum to assist the clinical pathology section while Dr. Cora Gilroy was on leave after welcoming a new son to her family. Thanks also go to Dr. Sandra McConkey and Dr. Andrea Battison who helped out during this time.
- Dr. Sandra McConkey, Dr. Shelley Burton and Ms. Ellen McMahon are planning to provide 2 wet laboratories on urinalysis at the Atlantic Provinces Veterinary Conference in Halifax on Saturday, April 24<sup>th</sup>. We hope to see you there!

## Staff Focus

### Ramona Taylor

*By Shelley Burton, Veterinary Clinical Pathologist and  
Andrea Bourque, Veterinary Anatomic Pathologist*

Achieving a pathologic diagnosis critically depends on proper tissue sectioning and staining. Pathologists at the Atlantic Veterinary College (AVC) are quick to note how fortunate they are to work with Ramona Taylor, our highly skilled chief histology technologist. Ramona is a Charlottetown native who went to Prince of Wales College and obtained her Registered Technologist diploma at the PEI Provincial Laboratory. She worked for 10 years in the field of microbiology before being

recruited to the AVC when it first opened in 1986. Since that time, she has accumulated tremendous experience in histologic processing and special stains. This alone is worth celebrating, but when combined with Ramona's cheerful personality, her incredible work ethic, her good communication skills and her attention to detail, it is indeed a powerful package. Her presence makes the AVC histology laboratory truly world class!



When asked what she most enjoys about her job, Ramona has a quick answer; she loves working with the pathologists and pathology residents. She enjoys new challenges and has excellent trouble-shooting skills; when a slide cut or special stain isn't perfect, she has the commitment and drive to repeat it until it truly is perfect! Histopathology slide preparation is not just a technical skill but an art and the AVC pathologists recognize that Ramona is a true artisan.

One of the pleasures of sharing the work environment with Ramona is that she is such an engaging person with many interests. She and her husband, Errol, have 3 grown daughters, Alana, Carolyn and Ellen, as well as 4 grandchildren. Both she and Errol are movie buffs who enjoy independent and foreign films. She is an avid gardener and reader, particularly enjoying British whodunits. Travel is high on her list of pleasures and she is looking forward to visiting New Zealand and Australia in 2010. Ramona grew up in a musical family; she is a skilled soprano and is considered one of PEI's best gospel music singers. In addition to gracing weddings and other events with her beautiful voice, she is a member of her local church choir, a member of the PEI Abegweit Chimes choir and the lead singer in a quartet named Taylor Made. This group will compete in a national competition in June 2010 in Wolfville, Nova Scotia – if you live in the area, be sure to watch for it!

**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@upepei.ca](mailto:cgilroy@upepei.ca)), Diagnostic Services, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.