AVC Diagnostic Services Laboratory at the 2014 Atlantic Provinces Veterinary Conference

By Cornelia Gilroy, Veterinary Clinical Pathologist

The Diagnostic Services Laboratory was represented at the annual Atlantic Provinces Veterinary Conference (APVC) in Halifax, Nova Scotia by Anatomic Pathologist Dr. Andrea Bourque, Clinical Pathologist Dr. Cornelia (Cora) Gilroy and Director, Ms. Liz Dobbin. This provided a great opportunity to visit with colleagues and friends, as well as receive feedback on our services. Thank you to everyone who invested time to stop by the exhibition booth! Diagnostic Services Laboratory was pleased to offer three draw prizes. Congratulations to our winners:

- First Prize of two complimentary surgical biopsies: Deborah White from Fundy Animal Hospital, Saint John, NB.
- Second Prize of two complimentary cytologies: Amanda Rowlands from Vetcetera Animal Hospital, Bedford, NS.
- Third Prize of one complimentary complete blood count, chemistry profile and endocrinology test: Dr. Katie Burrow from Kent County Veterinary Hospital, Richibucto, NB.

We look forward to seeing everyone again next year!

Figure 1: Our Diagnostic Services Laboratory booth at the APVC. Dr. Andrea Bourque and Dr. Cora Gilroy with Dr. Kip Grasse (centre) from Nova Scotia.
A ~6 month old mixed breed female dog was presented due to signs of hematuria and as a candidate for an ovariohysterectomy (OHE). The dog had been obtained as a rescue dog from Poplar River, Manitoba, and brought to Fredericton, New Brunswick, 5 weeks prior to presentation. During this period of ownership, the dog had been dewormed twice (Lopatol® and Sentinel®) as part of a routine preventative parasite program. Numerous bipolar plugged eggs were detected on urinalysis. The OHE was postponed until the verminous cystitis infection had been resolved and urine could be submitted to the Atlantic Veterinary College (AVC) Diagnostic Services Laboratory for further evaluation. At the AVC, the urine sample was found to contain numerous brown thick-walled bipolar plugged nematode eggs 60-80 x 39-46 microns in size (Figure 1). The embryo inside most eggs was in the 2-cell stage of development. The eggs were identified as *Dioctophyme renale*, the giant kidney worm.

There are two species of nematodes infecting the urinary system of dogs in North America and both have bipolar plugged eggs. In addition to *D. renale*, the other species is the capillarid, *Pearsonema plica* (formerly *Capillaria plica*). The eggs of the two parasites can be differentiated based on morphology and size. The eggs of *P. plica* are smaller (51-65 x 24-32 microns) in size, colorless and have a thinner shell wall relative to those of *D. renale*.\(^1\)

The natural definitive hosts of *D. renale* are mink and other wild mustelids.\(^2\) Infection in dogs occurs infrequently; infection in cats occurs rarely. A single kidney tends to be infected, usually the right one.\(^1\) Infections are acquired by the ingestion of intermediate hosts (water annelids through drinking) or paratenic hosts (freshwater fish or frogs).\(^2\)

The entire kidney is eventually consumed leaving only the capsule housing adult worms.\(^3\) All or some of the worms may be found free in the abdominal cavity in canine infections. Infections may be subclinical or result in hematuria and dysuria.\(^1\) The prepatent period is about 135 days; this dog therefore must have acquired the infection in Manitoba.\(^2\)

The parasite is not known to occur in Atlantic Canada. There are no anthelmintics known to be effective for treating dogs infected with *D. renale* and the treatment of choice is therefore nephrectomy.\(^3\)

Ultrasonographic evaluation indicated that the left kidney was normal and there was a lack of renal parenchyma in the right kidney. The dog underwent a nephrectomy and OHE. The right kidney was enlarged (grapefruit sized) and contained 8 adult worms (Figure 2). An additional 3 adult worms were recovered from the abdominal cavity. The worms were large (40 - 76 cm in length) and blood red in color. There were 3 male worms and 8 female worms recovered. The male worms were differentiated based on a smaller size (up to 45 cm in length) and the presence of the cup-shaped genital bursa at the posterior end (Figure 3). The dog recovered normally from the surgery. The worms were still...
Blood typing and Cross-matching: What’s the Difference?

By Dania Villarnivo, Veterinary Clinical Pathology Resident

Blood transfusions can be required in various situations: trauma with resulting hemorrhage, exposure to anticoagulant rodenticides, and immune-mediated hemolytic anemia are among the most common. Transfusions may be administered at a local veterinary clinic or at a secondary or tertiary referral centre. In either case, it is important that the blood product administered be compatible with the patient. An incompatible transfusion can cause significant adverse reactions, including hemolysis and anaphylactic shock.

The compatibility between a blood product (donor) and a patient (recipient) is typically tested with two methods, blood typing and cross-matching. These techniques are not only intended to minimize the risk of an incompatible transfusion but also aim to minimize immunizing a transfusion recipient against antigens they lack. Blood typing identifies the primary red blood cell antigens of the donor and recipient and this information is then used to ensure the recipient receives red blood cells with the same primary antigens. It is essential to note that although a donor and recipient share a common red blood cell antigen (blood type) they may have antibodies against other erythrocyte, leukocyte, platelet, or protein antigens that they lack. These antibodies can initiate an immunologic reaction with exposure to these antigens through a transfusion. Blood typing alone does not detect these antibodies. Cross-matching fills part of this gap by testing for antigen-antibody reactions that may not be related to the primary red blood cell antigens tested for with blood typing. A major cross-match involves mixing donor red blood cells with recipient serum while recipient red blood cells are mixed with donor serum in a minor cross-match. A compatible cross-match, however, does not completely eliminate the risk of a transfusion. A delayed reaction causing decreased red blood cell survival or a reaction to donor leukocytes or plasma proteins is not prevented. Blood typing and cross-matching should be used synergistically, not in replacement of one by the other.

In dogs and cats, blood typing is recommended prior to any red blood cell or plasma transfusion. Cross-matching should ideally also be performed before the administration of a transfusion in these species. Since dogs lack clinically significant naturally occurring antibodies, some authors suggest that the first blood transfusion is safe to administer without performing a cross-match to dogs with a known history. Cross-matching is especially recommended in canine patients with an unknown history since they may have potentially been recipients of blood products in the past. It is also recommended in patients who have had a transfusion given over 4 days prior as enough time would have elapsed for sensitization and antibody formation. Even if a recipient was previously blood-typed and cross-matched and found to be compatible with a given donor, this pairing can become incompatible in the future after a transfusion. In contrast to dogs, cats have clinically significant naturally occurring antibodies that can cause serious immune reactions. Cross-matching is thus essential in cats prior to any transfusion. Blood typing is usually not practical in horses due to the high variability of blood groups between individuals, the limited availability of donors, and the few laboratories performing the test. Cross-matching is particularly recommended when repeated transfusions are required for horses.

In recent years, the discovery of new red blood cell antigens has emphasized the importance of cross-matching in cats and dogs. In cats, the blood types A, B, and AB have long been recognized. However, feline recipients properly paired with donors with the same blood type can still be incompatible on a cross-match due to the presence of naturally-occurring antibodies in the recipient against the Mik antigen which may be present on donor red blood cells. Mik appears to be a high frequency antigen among domestic short-haired cats and anti-Mik antibody is capable of causing an acute hemolytic reaction. In dogs, the Dal antigen lacking in some Dalmatians was reported in the same year as the Mik antigen (2007). Dal was recognized as a separate antigen with no association with the previously known canine blood groups: Dog Erythrocyte Antigens (DEA) 1.1, 1.2, 3, 4, 5, or 7. Anti-Dal antibody can be produced in dogs lacking this antigen after exposure. The clinical significance of this antigen is still not known. The incompatibilities due to the Mik and Dal antigens were discovered only after performing cross-matching. Blood typing alone cannot detect incompatibilities due to these antigens. Administering a transfusion without previously performing a

References:
cross-match can therefore leave our patients at risk for a severe adverse reaction, especially in cats. There is the potential for some antibodies to target erythrocyte antigens that remain undiscovered.

Blood typing and cross-matching were originally developed as tube methods requiring expertise to perform and to interpret and were thus mostly reserved for specialized veterinary laboratories. Various methodologies have made these tests more accessible to general veterinary practitioners. Blood typing techniques in dogs (for DEA 1.1) and cats (for A and B) including the card agglutination assay (RapidVet®-H, Figure 1), the immunochromatographic cartridge method (Alvedia), and the gel column method (DiaMed) have been compared in several studies.\(^9\)\(^-\)\(^14\) These studies have shown that blood typing techniques adapted for in-clinic use are easy, reliable, and suitable for use in veterinary practices. Unfortunately, the gel column method which has a high sensitivity, specificity, and accuracy and can be adapted to detect several erythrocyte antigens in dogs including DEA 1, 3, 4, 7, and Dal, is no longer commercially available.\(^9\) Less information has been published for cross-matching techniques. A gel column agglutination major and minor cross-match method (RapidVet®-H) commercially marketed for use in dogs and cats has been independently evaluated in dogs by our group at the Atlantic Veterinary College.\(^15\) Our study found favorable results with canine samples (high specificity) although we were limited by the low numbers of incompatible pairings (sensitivity unclear) and occasionally came across results that were difficult to interpret. More recently, a chromatographic method (Alvedia) for cross-matching has become commercially available but has not yet been independently evaluated. The increasing availability of commercial blood typing and cross-matching techniques will help veterinary practitioners administer transfusions more safely while minimizing the risk of an adverse reaction.

In summary, pre-transfusion testing is an indispensable part of transfusion medicine aimed at minimizing adverse reactions and sensitization against donor antigens which can lead to severe reactions in the future. Blood typing and cross-matching are complementary yet separate methods that should be used together to identify the best donor blood product for a given patient. Commercial techniques for both blood typing and cross-matching are making it feasible for more veterinary practices to perform pre-transfusion testing and administer compatible blood products to their patients.

References:

Porcine Epidemic Diarrhea Virus (PEDV) and Diagnostic Testing

By Carmencita Yason, Veterinary Diagnostic Virologist

Porcine Epidemic Diarrhea (PED), caused by a coronavirus, was first reported in Canada in a pig farm in Ontario in January 2014. As of May 30, 2014, 62 farms have tested positive across Canada; 58 were in Ontario, 1 in Prince Edward Island (PEI), 1 in Quebec and 2 in Manitoba. Another coronavirus identified as porcine deltacoronavirus was detected in 6 farms in Ontario in March 2014.

The coronavirus causing PED belongs to the Family Coronaviridae, Genus Alphacoronavirus. The PED virus (PEDV) is distinguishable from the Porcine Transmissible Gastroenteritis Virus (TGEV) and Porcine Respiratory Coronavirus (PRCV) from the same family and genus. The porcine deltacoronavirus also belongs to this family but is in a different genus (Genus Deltacoronavirus). PED is not reportable federally but is now reportable in some provinces, including Alberta, Manitoba and Quebec. Recently, the Canadian Food Inspection Agency (CFIA) has reported PED to the World Organization for Animal Health (OIE) as an emerging disease based on OIE criteria. The other countries with similar outbreaks who have reported it as an emerging disease are the United States of America (USA), Mexico and Japan.

The disease was first reported in the USA in May 2013 and in Japan in October 2013. In the USA, the disease was called novel swine enteric coronavirus (SECoV) caused by two unrelated coronaviruses which are designated as porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV). The disease has now been reported in 29-30 states. PED was first reported in Europe in 1971 and became endemic in Asia in 1982. The PEDV infecting pigs in the USA, Canada and Japan has more than 99.4% homology to the PEDV strain which circulated in China in 2012. It is unknown how it came to the USA. Epidemiological investigation showed that the first 18 outbreaks in Canada, including that on the pig farm on PEI, were associated with swine feeds supplemented by PEDV-contaminated spray-dried porcine plasma. The subsequent outbreaks in Ontario, Quebec and Manitoba have been attributed to contamination through transportation, slaughterhouses and markets.

In a naïve farm, the introduction of PEDV often results in acute disease, characterized by watery diarrhea and vomiting. The morbidity can be 100% and the mortality can be as high as 100% in suckling pigs and 50-60% in weanling pigs. In older pigs (growers and sows), high morbidity but lower mortality is seen; clinical signs may include diarrhea, depression and anorexia. The virus can be spread via the oral-fecal route and through contaminated fomites such as boots and vehicles. PEDV does not affect people or pose any food safety concerns. A vaccine against PEDV is available but its efficacy has not been proven.

The diagnosis of PED is based on history, clinical signs, histopathology, electron microscopy and virologic tests. In the Regional Diagnostic Virology Services (RDVS) Laboratory, the diagnosis is confirmed by Real Time polymerase chain reaction (PCR) testing utilizing the protocol of the Animal Health Laboratory of the University of Guelph. We recommend using a special swab kit (swab plus viral transport medium) to collect appropriate samples such as feces, intestinal contents or environmental samples. Once collected, the swabs should be put in double plastic bags before they are shipped to the RDVS Laboratory. The molecular diagnostic test for detection of porcine deltacoronavirus is currently being developed and should be available soon.

In Atlantic Canada, as of May 30, 2014, PEDV has been detected only in 1 farm on PEI and the PEDV tests done on samples from other areas of Atlantic Canada are negative. The virus detected in PEI has 99.9 % homology to PEDV detected in Ontario and in the USA, and sequencing of the virus collected after 6-7 weeks did not show evidence of mutation. So far, no porcine deltacoronavirus has been detected in Atlantic Canada.

Please contact the RDVS at 902-566-0877 if you have questions related to PEDV testing, the special swab kit and/or the collection and shipment of PEDV specimens.
Specimen Submission for Diagnostic Bacteriology: What You Need to Know

By Jan Giles, Lorraine Lund, and Maria Vasquez, Veterinary Laboratory Technologists, Matthew Saab, MSc Student and Veterinary Public Health Technologist and Anne Muckle, Veterinary Clinical Bacteriologist

As veterinary clinicians, you take the first critical step in diagnosing patient illness. Despite good intentions, mistakes commonly made in the collection and submission of specimens for bacteriologic culture can adversely affect the diagnostic process and ultimately patient care. This article outlines key points in selecting, collecting, storing and shipping clinical specimens for bacteriological culture and susceptibility testing.

Ideally, specimens should be collected in the early stage of infection to increase the likelihood of isolating the primary pathogen(s) without overgrowth by other bacteria. Collecting specimens prior to antimicrobial therapy also makes it possible to start empirical therapy while culture and antimicrobial susceptibility testing occurs. Although there are no specific drug guidelines, we recommend a minimum of 7 days after completion of therapy to culture, otherwise bacterial growth may be inhibited and give inconclusive results.

A critical point is to collect the specimen from the actual site of infection. The more diligent you are in localizing this site, the more confidence can be placed in culture results. Collection of specimens directly from normally sterile anatomic sites (organs, body cavities) must avoid contamination by the resident flora of body surfaces such as mouth or skin, as well as by environmental or fecal contaminants. This can be accomplished by fine needle aspirate, centesis, venipuncture, transtracheal wash or bronchoalveolar lavage, catheterization, and the use of double guarded culture swabs. The use of strict aseptic technique when collecting specimens is also essential to minimize contamination of the collection site. A very common mistake is to swab the surface of wounds, or insert the collection swab into a draining tract. This usually produces a poor sample with mixed bacteria of questionable significance, and risks inappropriate antimicrobial treatment. Instead, the correct sample should be obtained from deeper tissues by fine needle aspiration or biopsy.

Tissue and fluid specimens are superior to material obtained with a swab. Surgically prepare the collection site surface (such as skin) prior to collecting aspirates or fluids. Collect an adequate volume of material as the laboratory will be using several types of culture media as well as direct stained smears. Transfer fluid samples into a sterile leak-proof plastic screw-cap container or a red-topped vacutainer blood collection tube. Other vacutainer types with anticoagulants such as EDTA are not suitable. Glass containers should not be used as these are dangerous to handle and ship. Needles are also a biosafety hazard, so needle aspirates must be transferred to either a sterile container or swab transport system. Broth transport systems, such as for blood or synovial fluid culture, should only be used when a single organism is likely to be involved and sterile collection technique has been used; a false positive growth can easily occur otherwise. Remember the biosafety of clinic and diagnostic staff by taking care not to contaminate the outside of containers and swab tubes. If contamination is suspected, clean the surface with a suitable disinfectant.

Collecting material with a sterile swab is convenient, but should not be used instead of fluid or tissue, as often there is not enough material obtained for optimal diagnostic testing. As an example, a few grams of feces are needed for *Salmonella* culture; a rectal swab is not adequate. Again, resist the urge to swab the surface of wounds or stick the swab into a draining tract. DO NOT use dry swabs; they are useless as bacteria dry out and die during transport! Swab transport systems containing buffered, non-nutritive salt solutions in a gel matrix, such as Amies medium, keep bacteria viable but not growing. There are several types of swab transport systems commercially available, including dual purpose swabs suitable for the recovery of aerobic and anaerobic organisms. Check the expiry date on the swabs; don’t take a chance using expired swabs. Charcoal medium swabs are not necessary and are unsuitable for making direct Gram-stained smears.

Label specimen containers, tubes and transport swab tubes directly with the patient and owner’s names, type of specimen and date of collection. Do not place the label on the outside plastic packaging of swab transport systems, as these are discarded in the laboratory. It is recommended to collect two swabs if you are submitting for aerobic and anaerobic culture. After collection, aerobic culture specimens should be stored in the fridge at ~4°C, but those for anaerobe culture must NOT be refrigerated. Instead, anaerobic specimens must be held at room temperature during storage and shipping. Milk samples must be chilled immediately to prevent bacterial overgrowth. Fill out a Sample Submission Form with patient signalment information (species, breed, sex, age), medical history and disease suspected, sample source, collection method, and whether there has been any antimicrobial treatment. Clearly indicate on the submission form if you are requesting anaerobic culture.

Diagnostic specimens should be shipped within 24-36 hours. Except for anaerobic culture samples, specimens can be refrigerated over the weekend if same day courier pickup is not possible and shipped packed along with ice packs (not
Here are some recent happenings in Diagnostic Services:

- Ms. Nancy Ingalls and Ms. Liz Dobbin attended the Veterinary Laboratory Association (VLA) annual meeting in San Diego, California, held April 4-5th. They were appreciative of the opportunity to meet with the VLA Board and the clients of the VLA Proficiency Program that Diagnostic Services produces to discuss recent changes to the program. Excellent presentations were given on topics such as the importance of red blood cell morphology; reproductive research, ecology programs, and veterinary diagnostics at the San Diego Zoo; fish hematology and microbiology and laboratory quality systems.

- Dr. Shelley Burton was recruited to join the American College of Veterinary Pathology (ACVP) Certifying Examination Committee for the newly implemented 2 phase testing in General Pathology due to her expertise and previous work with the ACVP.

- Ms. Jan Giles and Dr. Dave Groman attended the Eastern Fish Health Workshop in Shepherdstown, West Virginia from April 28th - May 4th. Both were also presenters as Ms. Giles presented "Validation of MALDI-TOF mass spectrometry for rapid identification of Yersinia ruckeri" and Dr. Groman presented "Exophiala angulospora causes systemic mycosis in Atlantic halibut (Hippoglossus hippoglossus)", a fungal pathogen isolated in our laboratory.

- The Canadian Animal Health Laboratorians Network (CAHLN) annual meeting was held in Ottawa, Ontario from June 1-4th. Representatives from Diagnostic Services included Dr. Carmencita Yason (Clinical Virologist) Liz Dobbin (Director, Diagnostic Services) and Maria Vasquez (Medical Laboratory Technologist).

- Mr. Matthew Saab represented Diagnostic Services at CAHLN and the American Society for Microbiology 114th General Meeting in Boston, Massachusetts from May 17-20th, 2014. At both meetings, he presented “Comparing traditional and selective culture methodology for the detection of methicillin-resistant Staphylococcus pseudintermedius in clinical canine specimens”.

- Congratulations to Dr. Barbara Horney, Veterinary Clinical Pathologist, Drs. Paul Hanna and Alfonso Lopez, Veterinary Anatomic Pathologists, Ellen McMahon, Medical Laboratory Technologist, and Lisa Cox, Administrative Professional. All were recognized for their commitment and valued dedication for 25 years of service to the University of PEI.

- Congratulations to Dr. Dania Villarnovo upon the completion of her clinical pathology residency in July. Dania is studiously preparing for the rigorous American College of Veterinary Pathologists Certifying Examination in September. All the best, Dania!

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**Specimen Submission for Diagnostic Bacteriology in Five Easy Steps**

1. Collect at early infection stage and prior to antibiotic treatment.
2. Collect only from the actual infection site and use sterile technique.
3. Collect tissue or fluid preferably, otherwise use swabs with transport medium, never dry swabs.
4. Label specimens directly and fill out a Sample Submission Form completely.
5. Store and ship specimens chilled, except for anaerobic specimens. Send to the laboratory within 24-36 hours properly packaged to prevent damage.

Transportation of Dangerous Goods Regulations Section 1.42 now applies to all diagnostic specimens, including those for bacteriology, parasitology and virology. This means that all diagnostic specimens can be shipped labelled as *Exempt Animal Specimen*.

Valid culture results depend on the quality of the specimens received. Damaged and leaking samples will not be processed. If you have questions regarding sampling, please refer to our laboratory manual or webpage. We are always glad to answer questions on the phone (902-566-0821) too!

**References:**

Staff Focus

Sarah Bernard

By Cornelia Gilroy, Veterinary Clinical Pathologist

Well known for her cheerful attitude, kindness and excellent skills, Sarah Bernard is a great asset to the histopathology laboratory at the Atlantic Veterinary College (AVC). Sarah, a native of New Glasgow, Nova Scotia (NS), earned her Bachelor of Science degree with a major in psychology from Dalhousie University in 1989. She subsequently attended the NS Community College Institute of Technology and obtained a diploma in Medical Laboratory Technology in 1993.

Sarah’s career as a registered medical laboratory technologist started after she moved to Bermuda where her husband, Kimball Bernard, a native of Prince Edward Island, was already employed as a chef. She worked at the King Edward VII Memorial Hospital in Bermuda as a chemistry technologist from 1993 until 1999. During her time in Bermuda, Sarah also taught Highland dancing and performed as a Highland dancer with the Bermuda Islands Pipe Band! Another joyous time in Bermuda was the birth of her daughter, Kara, in 1997.

Sarah and her family moved to Prince Edward Island in 1999 after which she devoted several years to her family. Sarah started working at the AVC Diagnostic Services Laboratory in the summer of 2006 after she became intrigued with an opening for a medical laboratory technologist there. Kara’s neighbor, a medical laboratory technologist employed at the time at the AVC, kept mentioning to Sarah that her skills were needed by the college! During her first year at the AVC, Sarah worked in both the bacteriology and histopathology laboratories. After this introductory year, she worked solely in the histopathology laboratory on a part-time (2007-2009) and then full time (2009 - present) basis and perfected her techniques in this area.

Sarah’s interests apart from work are many! Having put her dancing shoes to rest, she currently enjoys quilting, reading and is learning to knit. When not spending time or travelling with Kimball, now an instructor at the Culinary Institute of Canada or Kara, a grade 11 student, Sarah enjoys playing with Darcy, the family’s 5 year old Chihuahua dog. Sarah also is an active fundraiser for the Children’s Wish Foundation and supports the Canadian Cancer Society.

Submitting Fecal Samples

Please be advised that fecal samples submitted to the Parasitology Laboratory should be in containers that are sealed to prevent leakage. Plastic screw top containers inside of a zip lock bag are ideal. Due to the difficulty with disposal, we advise clients that glass containers are not to be submitted. Thank you!