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Diagnostic Update

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The Regional Diagnostic Virology Services at the Atlantic Veterinary College

By Carmencita V. Yason, Veterinary Diagnostic Virologist

The Regional Diagnostic Virology Services (RDVS) is a special entity of the Atlantic Veterinary College (AVC) Diagnostic Services, as it is a separate cost centre with unique functions. It was established in 1988 by the four Atlantic Canadian provinces through their provincial laboratories. The main objective of the RDVS was to provide advanced virology services in the Atlantic region, since the provincial laboratories did not have this service.

Clients of the RDVS include provincial laboratories, veterinary practitioners, veterinary pathologists, the AVC hospital, researchers of the AVC and other universities in Atlantic Canada. The clientele for aquatic virology includes practitioners and researchers in universities and research institutions in Canada and around the world. The infrastructure of RDVS has always been financed by the four Atlantic provinces through grant and user fees for mammalian food animal species, but Nova Scotia withdrew from the Regional Diagnostic Virology Agreement in April 2006. As a partner, AVC contributes one technical staff member and shoulders the overhead costs.

In this Issue:

The RDVS continues to respond quickly to the needs of our clients. The type of virology tests has gradually evolved from tedious and expensive cell cultures with long turnaround times to tests with fast turnaround times, such as negative staining electron microscopy, fluorescent antibody tests and ELISA tests. Since the RDVS was established, virology tests have been developed for bovine, porcine, avian, ovine, caprine, canine and feline species as well as for wildlife and aquatic species.

In the last 8 years, molecular diagnostic tests have been introduced by the RDVS. The tests available are polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), nested PCR, restriction fragment length polymorphism (RFLP) and nucleic acid sequencing. Very recently, the RDVS started implementing real time PCR technology. Compared to regular PCR, this new technology has faster turnaround times, is less prone to contamination, has more consistent results and can detect virus quantity in a specimen. The molecular diagnostic tests currently available are for important viruses of food animals, companion animals and aquatic species.

The renovation of the RDVS will start in October or November of 2007. A molecular diagnostic suite will be a major addition. We are currently working on grant proposals to provide additional equipment for the suite, as we are expecting expanded molecular diagnostic services once this suite becomes available.

The RDVS is currently a Canadian Food Inspection Agency-accredited laboratory for ELISA testing of Equine Infectious Anemia (EIA) and Enzootic Bovine Leukosis (EBL). It is currently working towards an International Standards Organization (ISO) 17025 level to maintain the accreditation of the stated tests and to establish compliance with testing requirements for foreign animal diseases such as Avian Influenza and Foot and Mouth Disease. In the last 19 years, the RDVS has collected reagents and cell lines and has developed the technical expertise to deal with investigation of disease outbreaks or the identification of emerging regional viruses and viral diseases.

The RDVS is the only service of its kind in Atlantic Canada. We are very grateful to the support from the Atlantic provinces and from our clientele and stakeholders during the past 19 years. Your strong continued support is needed in the maintenance of this important service in our region. The RDVS Website and Manual are currently being revised. The RDVS Fee Schedule is currently being prepared and will be mailed to you shortly. The RDVS will continue to communicate to you through the issues of this newsletter. If you have any questions, recommendations or suggestions, please contact:

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Canine Leptospirosis Statistics in Atlantic Canada

By Cora Gilroy, Veterinary Clinical Pathologist and Allan MacKenzie, Clinical Pathology Technologist

Leptospirosis is an important worldwide bacterial zoonotic disease. There has been an increased awareness of this disease by practitioners in the Atlantic Canadian region in recent years. Serum titres positive for leptospirosis can be due to exposure, vaccination or clinical disease.

The number of submissions to Diagnostic Services to test for leptospirosis titres peaked in 2005 (Table 1). In 2006, there were fewer seropositive and suspicious cases than observed in earlier years.

Table 1: Serological results for all canine Leptospira serovars

Year	Number of samples	Positive n (%)	Suspicious n (%)	Negative n (%)
2001	52	13 (25)	3 (6)	36 (69)
2002	48	20 (42)	14 (29)	14 (29)
2003	30	0 (0)	10 (33)	20 (67)
2004	60	17 (28)	19 (32)	24 (40)
2005	106	8 (8)	30 (28)	68 (64)
2006*	64	8 (13)	1 (2)	55 (85)

[1]Beginning in mid 2006, samples were submitted to a different external laboratory with only positive or negative results reported.

The highest percentage of positive and suspicious test results have been from New Brunswick (2004 and 2005) and Nova Scotia (2003 and 2006) over the past 4 years (Table 2). Provinces not listed for a particular year did not have any suspicious or seropositive cases.

Table 2: Percent positive and suspicious cases for individual provinces

Year	Province*	Positive† n (%)	Suspicious† n (%)
2003	NB	0 (0)	1 (3)
	NFLD	0 (0)	1 (3)
	NS	0 (0)	7 (23)
	PEI	0 (0)	1 (3)
2004	NB	13 (22)	14 (23)
	NS	4 (7)	4 (7)
	PEI	0 (0)	1 (2)
2005	NB	4 (4)	15 (14)
	NFLD	1 (1)	1 (1)
	NS	3 (3)	13 (12)
	PEI	0 (0)	1 (1)
2006	NB	4 (6)	0 (0)
	NS	4 (6)	1 (2)

*NB = New Brunswick, NFLD = Newfoundland, NS = Nova Scotia, PEI = Prince Edward Island

†Calculated based on the total number of submissions from all provinces per year

The frequency of seropositive and suspicious test results have been highest for *L. autumnalis* in 2003 and 2004 and for *L. grippotyphosa and L. bratislava* in 2005 and 2006, respectively (Table 3).

Table 3: Percent suspicious and seropositive cases to various *Leptospira spp.* serovars

Serovar	2003 %	2004 %	2005 %	2006 %
L. autumnalis	50	75	45	11
L. bratislava	30	44	34	56
L. grippotyphosa	40	53	50	44
L. pomona	0	22	8	33
L. icterohaemorrhagiae	30	25	11	22
L. canicola	10	17	29	11

Ranges:	Prior to 2006:	Suspect	1:80 - 1:160
		Positive	> 1:160
	2006*	Suspect	1:80 - 1:160
		Positive	> 1:160
		*Positive	> 1:50

*Note: Samples were submitted to a different external laboratory with only positive or negative results reported.

The *autumnalis* and *bratislava* serovars are not included in the current vaccines available against leptospirosis.

Increased titres to multiple serovars were noted in 50% (2003), 67% (2004), 37% (2005) and 33% (2006) of seropositive and suspicious cases. Multiple positive or suspicious titres are typically interpreted as being increased due to cross-reactivity, with the highest titre interpreted as being the infective organism.

Leptospirosis continues to be an important disease for veterinary patients in the Atlantic provinces. Appropriate testing is important for both patients and for epidemiological monitoring of this important zoonotic disease.

Soft Tissue Sarcomas

By Les Gabor, Veterinary Anatomic Pathologist

Whether it is a biopsy report relating to a dog or a cat, one of the most common diagnoses sent out to regional practitioners is that of a soft tissue sarcoma (STS). Pathologists are often accused of lumping inconvenient categories of disease into "garbage bag" categories – and in the case of STSs, this is a fair point. However, there is firm statistical and experimental evidence to support this practice. This short article summarizes pertinent clinical and diagnostic points regarding STSs.

What is a soft tissue sarcoma?

Metastatic rate	STS nomenclature
Low	
	Malignant fibrous histiocytoma
	Malignant nerve sheath tumor
	Hemangiopericytoma
	Leiomyosarcoma
	Mesenchymoma
Low to moderate	
	Fibrosarcoma
	Myxosarcoma
	Rhabdomyosarcoma
	Spindle cell tumor
	Liposarcoma

Table reference: Ehrhart N, Soft tissues sarcomas in dogs: a review; J Am Anim Hosp Assoc. 2005; 41:241-6.

There is quite a large group of tumors which previously had their own distinct diagnoses. Some brilliant histologists might still argue that certain cellular interlacing patterns and whorls (like some sort of histological ballet) suggest a certain cellular type. However, a number of studies using electron microscopy and immunohistochemistry have categorically showed that the morphology at a light microscopy level often cannot identify the exact tumor type. The assumption is, and I feel that it is reasonable, that many of these neoplasms arise from a precursor mesenchymal cell, which has the capacity to differentiate into many tumor types.

That being said, almost all of these neoplasms have similar clinical behaviors. The term "soft tissue sarcoma" therefore encompasses what we know about morphology, origin and likely clinical behavior. Importantly, however, the term has a different significance in cats versus dogs.

Incidence

Soft tissue sarcomas account for between 15-25% of our biopsy accessions at Diagnostic Services. Strictly speaking, these can occur in any soft tissue, but we most commonly see them associated with the dermis or subcuticular connective tissue. Most are solitary in both cats and dogs. They present as fixed masses involving the trunk, extremities or oral cavity. In cats, where there is anecdotal and epidemiological evidence linking some of these to vaccination or injection sites, the location is often near an injection site.

General prognostic points

Some key points to remember when a clinician receives this diagnosis for one of their feline or canine patients:

1) Soft tissue sarcomas have poorly defined histological margins and often infiltrate into the fascial planes. They are invasive and destructive.

2) Therefore, they are difficult to resect and typically need wide margins of excision. Margins of 2-3 cm are typically advised. A figure to quote for owners is a disease free median post surgical survival of approximately 700 days.

3) Recurrence is common, and owners should be warned of this. In a recent paper, the recurrence rate was 15%. There is very little evidence to support the use of either adjuvant chemotherapy or radiotherapy.

4) The rate of metastasis is low (10% in one recent study), especially in cases where excision has been complete.

5) There is some evidence that feline vaccine associated sarcomas have slightly higher rates of metastasis and behave in a more aggressive fashion. As in dogs with STSs, there have been only modest and likely insignificant results of using adjuvant chemotherapy or radiotherapy. Survival without resection has been reported at approximately 120 days, but improves to 570 days with resection and 840 days with adjuvant radiotherapy. A good overview of feline vaccine associated STSs is found in the following JAVMA issue: Morrison WB et al. 2001; 218(5): 697-702.

Summary

A diagnosis of STS is extremely common in small animal practice. The cell of origin is uncertain but biological behavior is predictable. The tumours are locally invasive, destructive and have a tendency to recur following resection. Despite this, excision with wide margins is the mainstay of treatment. In dogs, the median disease free survival time following resection is approximately 700 days, but is closer to 570 days in cats. Other complimentary therapies such as chemotherapy and radiotherapy are still considered questionable.

New Chemistry Analyzer

By Pam Maloney, Medical Laboratory Technologist

Diagnostic Services has recently upgraded its clinical chemistry analyzer from a Hitachi 917 to a Cobas 6000. New features of the Cobas 6000 include clot detection, carryover protection, Smart Track Rotor and STAT port, and reagent loading during operation.

By means of the special wash function and by ultrasonic mixing stations, a reduction of carryover of samples is achieved. The patented ultrasonic mixing stations are non-contact with reagent or patient samples.

The Smart Track Rotor consolidates clinical chemistry testing, immediately routing sample racks for routine, re-run, and STAT samples. With the newly available STAT port feature, the Cobas 6000 provides quicker turnaround time and reporting for high-priority samples. Samples entering the STAT port go directly to the Smart Track Rotor for processing within 60 seconds of entry.

Sample loading of up to 150 samples in five-position racks is continuous. Change over of reagents is automatic, as pre-calibration of the second lot occurs while the first lot is running.

Reagent packs are ready to use which reduces preparation time. The reagents are common to our existing reagents on our Hitachi 917, allowing seamless transfer of results and reference intervals. These improved features allow improved delivery of results to our clients.

Quality Assurance – Monitoring Your In-house Test Results

By Cora Gilroy, Veterinary Clinical Pathologist

Quality assurance, including assessment of accuracy and precision, is vital for laboratory testing. Quality assurance needs to be applied to the **entire test process** including sample collection, transport, storage, analysis and reporting the data. Quality control refers to the techniques and procedures that monitor the performance parameters for a particular test.

Internal and external quality control procedures are both critical. One important aspect of internal quality control includes the use of controls, which are often available from the manufacturer of the analyzer. Proper handling, shelf life and storage requirements for the controls must be strictly adhered to, as deviations from accepted protocol may invalidate the results. Controls are used to verify the accuracy of the calibration and the stability of the system. An important aspect of external quality control is comparing results from your in-house test or analyzer with results obtained from a reference laboratory. The

sample submitted for comparison must be obtained at the same time and handled in the same manner as the one used for in-house testing. One practical way to accomplish this is to send AVC Diagnostic Services a duplicate sample for the various tests performed in your clinic on a regular basis. Sending sample(s) once a month is a good way to embark on an external quality control program.

A structured external quality assurance program (Veterinary Laboratory Association Quality Assurance Program) is also available provided by Diagnostic Chemicals Limited (DCL) in conjunction with Diagnostic Services. If interested, please contact Stacie Hotham at 1-800-565-0265 or vqap@dclchem.com. Information is also available on the website for DCL (www.dclchem.com).

PFA-100 Analyzer

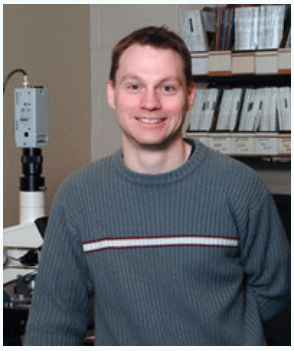
By Linda Ruschkowski, Medical Laboratory Technologist

We have recently acquired a platelet function analyzer called the PFA-100. Assessment of platelet function has traditionally been crude or available only in specialized research laboratories. This situation may improve with the introduction of the PFA-100 analyzer. Our clinical pathology resident, Dr. Noel Clancey, is working on a project to assess platelet function in sick dogs as well as evaluating interference effects in the analyzer from anemia. The analyzer has already been helpful in identifying several dogs with von Willebrand's disease. More work needs to be done before we know if this analyzer will be routinely available to local veterinarians or not.

Staff Focus

Clinical Pathology Resident

By Linda Ruschkowski, Medical Laboratory Technologist



Dr. Noel Clancey joined us in August of 2005 to pursue a 3 year combined Master of Veterinary Science and Residency in clinical pathology. His research project involves evaluating a point-of-care platelet function analyzer for use in dogs. Noel is originally from the outskirts of Dartmouth, Nova Scotia. He graduated from the AVC in 1999 and practiced small animal medicine and surgery in Dartmouth at the Colby Village Veterinary Hospital for a short time. After travelling to western Canada, Noel joined the Granville Island Veterinary Hospital in Vancouver as a clinical associate in surgery. After six years of private practice, Noel was ready for another challenge and he is enjoying his training in clinical pathology. When not busy studying or bounding through the laboratory with his quick Irish step, Noel has a passion for photography, travel and playing the guitar. He also enjoys the outdoors and loves hiking, kayaking, mountain biking and skiing. He shares life in Charlottetown with his beloved 3 legged cat, Hobbes.

Locum Clinical Pathologist

By Shelley Burton, Veterinary Clinical Pathologist

In June, we were pleased to welcome Dr. Jim Bilenduke as a clinical pathologist, who will be with us until December of 2007. He temporarily takes the place of Dr. Cora Gilroy, who recently welcomed a new baby to her family. Jim was born in Winnipegosis, Manitoba, but grew up in British Columbia. Following his pre-veterinary program at the University of Manitoba, he was accepted into the Western College of Veterinary Medicine (WCVM). After obtaining his veterinary degree in 1986, he enjoyed small animal practise in Calgary and Toronto. He returned to the WCVM in 1989 and completed his residency and Master of Veterinary Science program 3 years later. Jim established the Calgary branch of Central Laboratories for Veterinarians in 1992 and worked there until he joined the Langley, British Columbia branch in 2002; his work continued at that site until March of 2007. He became board-certified by the American College of Veterinary Pathology in 1993. Dr. Bilenduke has a wealth of diagnostic experience and a special interest in the hematology of uncommon species. In his free time, he enjoys life in Vancouver with his wife, Dr. Margie Scherk, an AVBP boarded feline practitioner, and their 5 cats. His interests include travelling and enjoying specialty beers.

Laboratory News

Client Survey

Diagnostic Services would like to thank those who participated in our client survey sent out in February 2007. It is our goal to improve client services and your feedback is instrumental in this process. We value your opinions!

Diagnostic Services Handbook

We are currently updating our Diagnostic Services handbook. It will provide accessible information concerning tests available at our laboratory, sample requirements and sample handling. Information concerning specialized tests sent to external laboratories will also be included. We are hopeful copies will be available in the fall of 2007.

Reader Feedback: The ***Diagnostic Update*** group invite comments or suggestions for future topics in the newsletter. Please submit your comments to *Ms. Linda Ruschkowski* (ruschkowski@upei.ca), Diagnostic Services, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.