UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

TSE testing at the AHL

Testing for the transmissible spongiform encephalopathies (TSE's) is topical for many - veterinarians, livestock producers, game farmers, wildlife managers, and consumers. The AHL is approved by the Canadian Food Inspection Agency as a member lab in the Canadian TSE Laboratory Network. As an approved member, we are able to obtain and use the BioRad ELISA kit for testing for bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD), and scrapie. We were audited in September for scrapie resistance PrP genotyping, have met all of the requirements of ISO 17025, and expect to be fully accredited in early 2006.

Details of samples to send, fees, etc. are posted in a LabNote on our website at http://www.uoguelph.ca/ahl/LabNotes1/LabNote7_TSE.pdf

Histopathology service

Pathologists within the AHL labs at Guelph and Kemptville read the histopathology on their own necropsy cases. Although mail-in biopsy/necropsy cases are all sent to Guelph for cutting and staining, slides from mail-in cases are distributed among available pathologists at both labs. Should you wish to contact Drs. Jan Shapiro or Mirjana Savic in Kemptville, we have added a **toll-free telephone number to Kemptville** for your convenience **877 687 5422 .**

Ovine scrapie PrP genotyping and flock certification programs

The AHL is one of the laboratories offering this service. If your ovine clients are asking about these programs, please consult scrapiecanada.ca

AHL Christmas hours, 2005

Between Christmas and New Year's this year, the AHL will be open with limited staffing and offering limited services 9 AM to 5 PM, **December 28, 29, 30 (Weds, Thurs, Friday).** Emergency necropsy service is available and Specimen Reception is staffed in the Guelph lab on weekends and holidays except for Christmas Day, Dec. 25 (labs closed) - our specimen drop box is available 24/7.

If you would like to **schedule testing** over this period, pleased call by Dec 12. If you have testing that must be done urgently over the holidays, **please call the lab to determine the availability of the urgent testing that you require**. We will do our best to oblige you and your clients.

Please note that the last day for shipping samples to US laboratories will be Weds. Dec. 21/05. Shipments to the US will resume Monday Jan. 2/06.

Season's Greetings from the staff of the Animal Health Laboratory

Accreditations, registrations, proficiency testing - what's in it for you?

Grant Maxie

The AHL, like all reputable labs, takes great pride in serving clients well through producing quality results. We do everything possible to ensure high quality client service through implementing a **quality system**.

The basis of any quality system is "*say what you do, do what you say, and prove it*". Emphasis is placed on documentation - if you didn't write it down, it didn't happen! A **quality manual** is essential, and includes standard elements - quality policy statement, organizational structure with responsibilities and authorities, staff training, test selection and validation, sample handling, purchasing, equipment maintenance, quality control of tests, measurement traceability, control of nonconforming testing, and corrective and preventive actions. Written *standard operating procedures (SOP's)* govern all testing activities. Of course, the objective of a quality system is not only to ensure quality results, but to also lead to **continuous improvement**.

Although labs typically do internal audits, the "prove it" component of the quality system is also best assessed by external third-parties.

- **AAVLD accreditation.** We are visited at least once every five years by a site visit team from the American Association of Veterinary Laboratory Diagnosticians and undergo an extensive peer review. The AHL received full accreditation in 2005, after our third successful AAVLD audit.
- **ISO 9001:2000 registration.** As part of the Laboratory Services Division, our overall quality management system is audited annually; we successfully passed the audit in Sept/05.
- **ISO 17025 registration.** We also underwent assessment this fall by an ISO 17025 team. This audit encompasses the elements of ISO 9001, but also specifically examines individual tests to ensure competence of testing. The Division has about 150 tests accredited to the ISO 17025 standard within its *scope of accreditation*. A new test - our scrapie resistance PrP genotyping test - was successfully audited this fall, and we expect accreditation to be announced in early 2006.
- Other accreditations held by the AHL:
    - **OFA** (Orthopedic Foundation for Animals)
    - **CFIA** (Canadian Food Inspection Agency)
    - **CAEAL** (Canadian Association of Environmental Analytical Laboratories)

A key means of maintaining a highly functional quality system is **external proficiency testing**. We participate in a broad range of programs in order to assure ourselves and our clients that we are meeting industry standards, e.g., VLA (Veterinary Laboratory Association - biochemistry, hematology, pharmacology, endocrinology, serology), Randox RIQAS (biochemistry), Michigan State University (endocrinology), Bayer (urinalysis), USDA/NVSL (Johne's disease).

Through our annual client questionnaire, monthly service checks, and personal contacts, we are receiving **client feedback** that these efforts are worthwhile! *AHL*

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Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends.

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

Immunohistochemical tests available at the AHL

Josepha DeLay

The AHL currently has immunohistochemical (IHC) tests available for **13 infectious agent antigens and 23 cell markers** (Table 1). We also offer panels of tests for porcine respiratory disease (PRRSV, PCV2, influenza A virus) and bovine abortifacient agents (IBRV, BVDV, leptospires). Cell marker identification is used primarily to determine the histogenesis and, correspondingly, the predicted behavior of anaplastic tumors.

IHC techniques exploit antigen-antibody reactions to identify infectious and cellular antigens within formalin-fixed tissue sections. One of the major advantages of immunohistochemistry in the diagnosis of infectious disease is the ability to visualize antigen presence in association with histologic lesions induced by the infectious agent. Use of formalin-fixed samples also facilitates preservation of tissue that would normally deteriorate during transport to the laboratory and preclude other testing, such as with many gut-associated pathogens (e.g., TGEV).

In most circumstances, the coordinating pathologist will recommend IHC if deemed beneficial to the case. If you have questions about IHC testing and applications, please contact Dr. Josepha DeLay at 519-824-4120 ext. 54576, jdelay@lsd.uoguelph.ca. *AHL*

Table 1. Current AHL immunohistochemical tests, December 2005

Infectious agents	Cell markers
Bovine viral diarrhea virus (BVDV)	Actin: smooth muscle actin, muscle actin
Bovine coronavirus	CD3
Bovine herpesvirus-1 (Infectious bovine rhinotracheitis virus, IBR)	CD79a
Canine distemper virus	CD18 (canine, feline)
Feline coronavirus / FIP (under validation)	Chromogranin
Influenza A virus	Cytokeratin HMW
Leptospira spp.	Cytokeratin AE1/AE3 (pancytokeratin)
Mycoplasma bovis	Cytokeratin 7
Porcine circovirus type 2 (PCV2)	Desmin
Porcine reproductive and respiratory syndrome virus (PRRSV)	Factor VIII-related antigen
Transmissible gastroenteritis virus	Glial fibrillary acidic protein (GFAP)
Toxoplasma gondii	Hepatocyte paraffin 1 (Hep Par 1)
West Nile virus (monoclonal / polyclonal)	Lambda light chains
Porcine respiratory panel: PRRSV, PCV2, Influenza A virus	Mast cell tryptase
Bovine abortion panel: BVDV, IBRV, *Leptospira*	Melan A
	Neuron-specific enolase (NSE)
	S100
	Synaptophysin
	Thyroglobulin
	Uroplakin
	Vimentin

AHL Lab Reports

RUMINANTS

Taxus spp. toxicosis in a herd of cattle

Murray Hazlett, John Leonn, Peter Lusis

A herd of about 30 pastured beef cattle experienced unexpected overnight deaths of 3 animals, with another dying the next morning. Dead cattle weighed between 200 and 400 kg, two female and two male. The pasture was not conducive to bloat, but there was some concern with spraying of a neighboring field with pesticides the previous week. The cattle had access to a burning area on which a quantity of evergreen shrubs and pear-tree clippings were dumped the previous day.

The 4 animals were submitted to the AHL for necropsy, as well as 2 of the shrubs for plant identification. (Fig. 1) The shrubs were identified as *Taxus* spp. (probably Japanese yew). The necropsies revealed no significant lesions, all animals were in good body condition, and **yew branches with needle-like flattened leaves were found easily in rumen content of all 4 animals** - estimate of 300-400 g per animal. No yew was found in oral cavity or abomasum. Histology done on 2 of the animals was unremarkable. *Taxus* (yew) toxicosis was diagnosed. The animals were removed from the area and there were no further losses.

There are several species of *Taxus* in Ontario. *T. canadensis* is a low-lying shrub found in wooded locations. Of the non-native species, only Japanese yew (*T. cuspidata*) is common, and it appears as a shrub or small tree. It is sold in nurseries for landscaping, especially for hedges and foundation plantings, and can tolerate cold conditions as well as both sunny and shady locations. They typically form small red berry-like arils around the seed. The toxicity of *T. canadensis* appears to be relatively low, and moose and deer will graze on it with impunity. In contrast *T. cuspidata* may have much higher levels of taxines, in particular taxine B, contained within its seeds, needles and stems, but not within the aril. The toxin seems to target myocardial conduction, possibly by inhibiting Na and Ca currents or potassium channel effects. *AHL*

Figure 1. *Taxus* spp. leaves, close up.

Reference

Burrows, GE, Tyrl, RJ. Toxic Plants of North America. Iowa State University Press, ed 1, 2001:1149-1154.

Milk sample vials and boxes

Jim Fairles

Other than virus transport medium, swabs, and formalin jars, which are available gratis through the AHL, most supplies necessary for submission of samples are available through Veterinary Purchasing Company and CDMV. Traditionally the AHL has also provided milk sample vials for submission of milk samples. Due to ongoing monetary constraints in the subsidized mastitis testing service, it has become necessary to charge for these vials.

Until current stocks are used up, milk sample vials will continue to be available through the AHL. An order comprises a box of 12 milk sample boxes with 20 vials in each (240 vials) with the associated submission forms for **$72.00** ($0.30 per vial). Once our supplies are exhausted, we will make sure that these are available through your veterinary purchasing supplier. *AHL*

POULTRY

Cholangiohepatitis in broiler chickens

Brian Binnington, Emily Martin

During 2005, the AHL received samples of broiler chicken livers from carcasses that were condemned during processing due to hepatitis. Samples were also submitted from broilers that had suspected hepatitis during the grow-out period. The majority of the samples were sections of liver submitted in 10 % formalin for histopathology; only a few samples of fresh liver suitable for bacterial culture were submitted.

The fresh livers were firm with rounded edges, pale yellow-orange to green-brown with scattered red or tan foci and a prominent lobular pattern. On microscopic examination, the inflammatory reaction was centered on portal areas with moderate to marked accumulations of mixed inflammatory cells, including heterophils, myelocytes, lymphocytes and plasma cells. Light to severe fibrosis and biliary ductal hyperplasia were present in portal areas and extended into the surrounding sinusoids. The degree of hepatocyte atrophy increased with increasing severity of fibrosis and biliary hyperplasia. In some livers, only clusters of atrophic hepatocytes were embedded in fibrous tissue and hyperplastic bile ducts. Scattered granulomas with macrophages and giant cells surrounded pooled bile and cell debris. In a small number of liver sections, scattered bile ducts contained cell debris and plump gram-positive rod-shaped bacteria (Fig.1). In 3 cases, liver samples were suitable for bacterial culture. Small to large numbers of *Clostridium perfringens* were isolated in 2 cases, occasional to moderate numbers of *Escherichia coli* in 3 cases and occasional *Escherichia fergusonii* in 1 case.

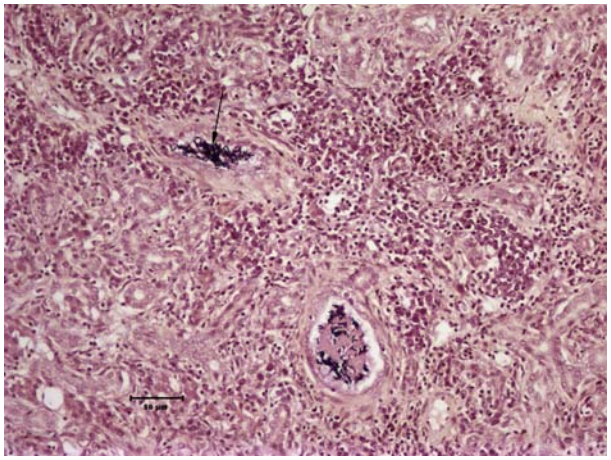

Figure 1. Cholangiohepatitis with intraductal gram-positive bacteria. Inflammatory cell accumulations, biliary hyperplasia and fibrosis are present.

Liver lesions in broiler chickens that are compatible with cholangiohepatitis, as previously described in the literature, are seen annually but infrequently at the AHL. **During 2005, there have been increased submissions of cholangiohepatitis livers from carcasses condemned at slaughter**. Suggested causes of these liver lesions have included inclusion body hepatitis (IBH), mycotoxins or bacterial infection and/or toxemia. In the AHL cases, only 1 submission in 34-day-old birds had concurrent IBH and mild cholangiohepatitis in the flock. The remaining cases had no lesions suggestive of IBH. The majority of the submissions had little or no flock history, however, in several cases there was a history of necrotic enteritis during the grow-out period. Evaluation for mycotoxin exposure was not possible because of the types of samples that were submitted.

There has been repeated association of *Clostridium perfringens* enteric infections and the occurrence of cholangiohepatitis at slaughter. Experimentally, cholangiohepatitis has been reproduced by bile duct ligation or inoculation of *Clostridium perfringens* into the bile ducts. The presence of cholangiohepatitis lesions at slaughter has been shown to be an indicator of subclinical and clinical necrotic enteritis during the grow-out. *Clostridium perfringens* associated hepatitis can be associated with severely impaired production performance.

The incidence of *Clostridium perfringens* associated necrotic enteritis and cholangiohepatitis may be increasing due to restrictions on the in-feed usage of antimicrobial compounds that may have benefits in the inhibition of subclinical *Clostridium perfringens*-associated diseases. *AHL*

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Lovland A, Kaldhusdal M. Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immun Med Microbiol 1999;24:345-351.

Lovland A, Kaldhusdal M. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens*- associated hepatitis. Avian Pathol 2001;30:73-81.

SWINE

Porcine circovirus type 2 - associated disease continues to increase

Susy Carman, Beverly McEwen, Josepha DeLay, Hugh Cai, Jim Fairles, Tony van Dreumel

***Porcine circovirus* type 2 (PCV2)-associated disease continued to increase over the summer of 2005**, compared to the previous 7 years (Fig. 1), increasing from 6% of total swine cases submitted in June to 8.5% of cases in September.

The PCV2 PCR-RFLP typing for all PCR testing requests continues to show a significant change from RFLP type 422 to RFLP type 321 (Fig. 2), with an increase in RFLP type 321 from 40 in June to 80 in September. These changes in RFLP typing patterns reflect a consistent change in the gene sequence recognized by two restriction enzymes. When 4 Ontario RFLP type 321 viruses were sequenced, they were all found to have greater than 99% sequence homology with each other, and to have 98% homology with those reported for the UK, France and China. These 4 Ontario viruses had only 91.6% sequence homology to an Ontario RFLP 422 virus and were only 92-93% similar to those reported from the USA. *AHL*

References

DeLay J, et al. Porcine circovirus 2-associated disease is increasing. AHL Newsletter 2005:9;22.

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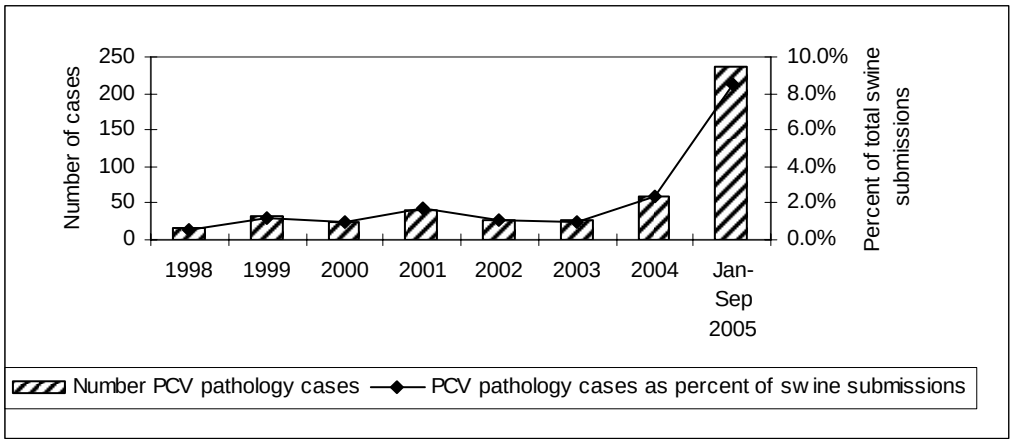

Figure 1. Number of PCV2 pathology cases and percent of PCV2 pathology cases of total swine submissions.

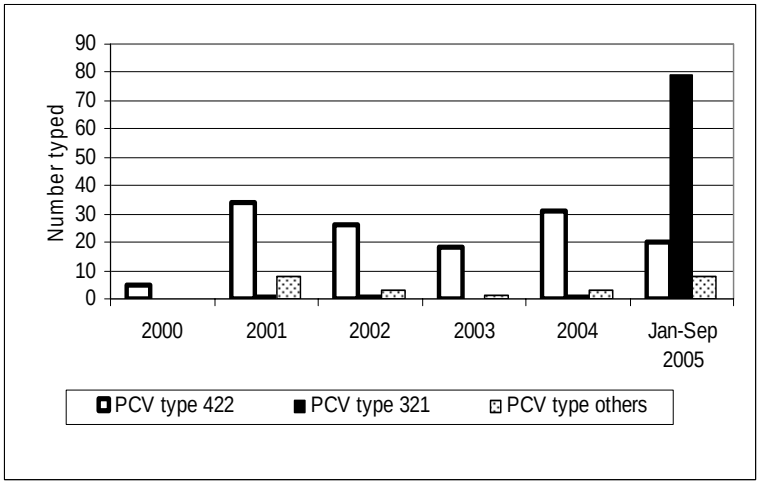

Figure 2. PCR-RFLP typing of PCV2, 2000-2005.

PRRSV outbreak in Ontario declines

Susy Carman, Beverly McEwen, Jim Fairles

In the Dec 2004 and the June 2005 AHL Newsletter, we presented information on the 2004-2005 PRRSV outbreak in swine herds in southwestern Ontario. The updated epidemic curve shows the outbreak declining from a high of 55% of all cases testing positive for PRRSV by PCR in Oct-Dec 2004 to **39% of cases in July-Sept 2005** (Figure 1).

The total number of cases submitted to the AHL for PRRSV diagnosis using PCR decreased from 396 in the Apr-June 2005 interval to 297 for the July-Sept 2005 interval, with the total number of cases found to be positive also decreasing from 194 to 117. These data do not include monitoring cases, where semen was the sample submitted. *AHL*

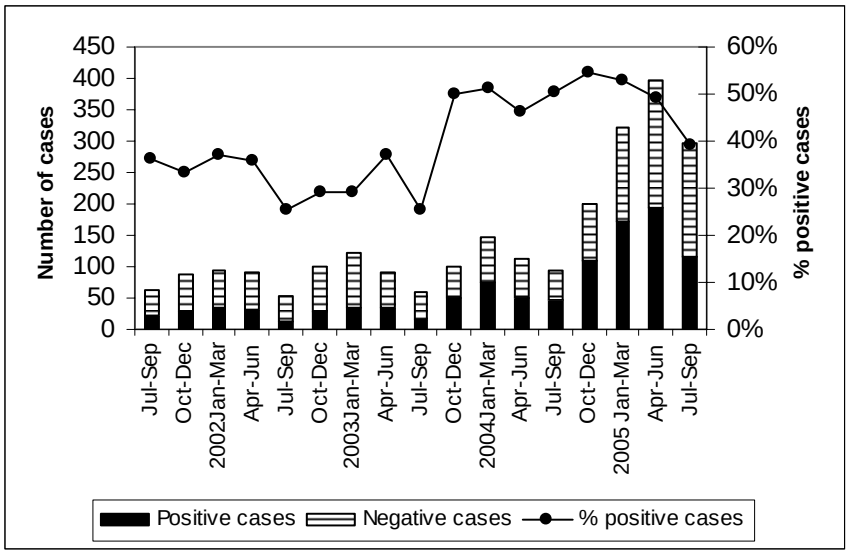

Figure 1. PRRSV PCR submissions tested by the AHL April 1, 2001- September 30, 2005, with semen monitoring removed.

Swine influenza virus H3N2 Ontario/05 serology now available at the AHL

Susy Carman

The AHL routinely offers hemagglutination-inhibition (HI) assays for H1N1 Ontario/81, H1N1 North Carolina/01, H3N2 Colorado/77, and H3N2 Texas/98 to test "paired sera" for antibody to swine influenza viruses to establish a serological diagnosis of disease. We now also offer an HI assay using the triple reassortant H3N2 virus recovered during the outbreak in Ontario swine in the spring of 2005, designated H3N2 A/Swine/Ontario/05. This is the best test for the assessment of paired sera for the diagnosis of disease due to this new virus.

Since there is no check-off box for this test on our current submission form, please write your request in the history section of the submission form. Routine monitoring to assess response to vaccination using single sera should continue to be evaluated using the IDEXX H3N2 ELISA serology test. *AHL*

HORSES

Ontario Racing Commission Death Registry: interim summary of necropsy diagnoses for 2005

Josepha DeLay

Since 2003, the Ontario Racing Commission (ORC) has required that all racehorse deaths be reported if the horse has died or been euthanized within 60 days of racing or qualifying in Ontario, and the necessity for a necropsy is then determined. In 2003 and 2004 respectively, 125 and 141 necropsies were performed at the Animal Health Laboratory on horses identified by the Death Registry. As of September 30, 79 ORC necropsies have been conducted in 2005. This program continues to provide excellent data regarding the causes of morbidity and mortality in Ontario racehorses, and supports research to improve animal health and welfare in the racehorse industry.

Of the 79 horses necropsied to date in 2005, 44 (56%) were Standardbreds and 35 (44%) were Thoroughbreds. Overall, the causes of death or euthanasia were similar to those in previous years, with similar distribution of these diagnoses in the affected population (Table 1).

Fractures remained the most common cause of death or euthanasia, with 36 (46%) animals diagnosed with limb fractures and 3 (4%) with severe fractures at other sites. Non-fracture diagnoses were varied and often single events, and involved primarily the gastrointestinal, cardiopulmonary, and nervous systems. *AHL*

(continued on p. 32)

ORC necropsy diagnoses, 2005 *(continued from p. 31)*

Table 1. 2005 ORC Death Registry necropsy diagnoses to Sept. 30

Fractures, 39 (49%)		**Non-fracture diagnoses, 40 (51%)**			
Limb fractures	**35 (44%)**	Gastrointestinal accident	5	Entrapment	1
P1	8	Pulmonary hemorrhage	3	Endotoxemia	1
Metacarpal 3	6	Colitis	2	Equine protozoal myelitis	1
Sesamoid	5	Encephalitis/myelitis	3	Cecal rupture/peritonitis	1
Carpus	5	Undetermined cause of death	4	Chordae tendineae rupture	1
Metatarsal 3	4	Arthritis	2	Hematoma	1
Tibia	4	Pulmonary edema/congestion	1	Aspiration pneumonia	1
Humerus	2	Limb/tendon laceration	1	Hepatopathy	1
Radius	1	Peritonitis/gastric rupture	1	Acute cerebral hemorrhage	1
		Suspensory ligament rupture	1	Epiglottic entrapment	1
Other fractures– pelvis, skull, rib, vertebral	**4 (5%)**	Aortic rupture	1	Exertional rhabdomyolysis	1
		Colonic venous infarction	1	Endocardial fibroelastosis	1
		Hemolytic anemia	1	Severed jugular vein	1
		Lightning strike	1		

COMPANION ANIMALS

Canine influenza virus

Susy Carman

Canine influenza virus (CIV) has been reported as a cause of kennel cough in family dogs in Florida and New York State, with antibody found in the serum of dogs from other regions of the USA. CIV is in the genus *Influenzavirus A*, and is a derivative of equine influenza virus-2 (H3N8), which has not been demonstrated to infect humans.

Since this is a new agent in dogs, all dogs are susceptible to infection. Morbidity in shelters can be 80%, with nasal discharge, increased respiratory rate, fever and persistent cough for 2-3 wk. Mortality may be decreased to 5% by antimicrobials, with death due to hemorrhagic pneumonia.

The AHL offers tests for CIV infection, as it does for other causes of kennel cough that also need to be considered in the differential diagnosis. More information, and the tests offered for virus detection and serology for CIV, is available on our website at http://www.uoguelph.ca/ahl/ . When dogs are presented late in infection, paired serology is the suggested method of diagnosis.

Although testing is ongoing, we have not yet identified any cases of canine influenza virus infection. *AHL*

Diagnosis of canine hypothyroidism

Brent Hoff

Diagnostic approach to screening for hypothyroidism:

- A normal TT4 will preclude hypothyroidism in 90% of cases.
- To detect all (99%) hypothyroid dogs, use TT4, TSH and fT4D (BEST SENSITIVITY).
- If you find no breed, drug, age, non-thyroidal illness (NTI), or other confounding factor (e.g., glucocorticoids, sulfonamides, phenobarbital, carprofen, clomipramine) - low fT4D, high TSH (BEST SPECIFICITY).
- If there is concurrent NTI, or confounding drugs: fT4D and TSH +/- TgAA is *preferred.*

Interpretation:

- Normal fT4D and TSH almost always identifies a euthyroid animal.
- A low TT4 or fT4D with a high TSH confirms the diagnosis in most cases.
- A positive TgAA is most valuable to support abnormal TT4, fT4D, and/or TSH

TT4 = total T4; TSH = canine thyrotropin; FT4D = free T4 by equilibrium dialysis; TGAA = thyroglobulin autoantibodies *AHL*

Newsletter Figures are posted in full color in the Web version of the Newsletter - http://ahl.uoguelph.ca