UNIVERSITY of GUELPH
LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Dr. Maria Spinato joins the AHL

Dr. Maria Spinato was the successful candidate in our recent competition for an AHL veterinary pathologist.

Dr. Spinato graduated from OVC in 1985, earned a DVSc in anatomic pathology in 1989, was board certified by the American College of Veterinary Pathologists as an anatomic pathologist in 1990, and earned an MBA from the University of Regina in 2004. She was most recently manager/pathologist in the Abbotsford BC Animal Health Ctr, and was previously a pathologist and then manager in the Regina lab of Prairie Diagnostic Services (formerly Saskatchewan Agriculture and Food) from 1989-2005.

Maria brings with her tremendous depth and breadth as a client-focused diagnostic pathologist, and we are delighted to have her join our team this fall.

AHL Christmas hours, 2007

- Limited testing services 0900-1700, Dec 24, 27, 28, 31, in both Guelph and Kemptville.
- Emergency necropsy and Specimen Reception are available in the Guelph lab on weekends and holidays except for Christmas Day, Dec. 25 (lab is closed) - our specimen drop box is available 24/7.
- Please call if you would like to schedule testing over the holiday period.

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

The effects of blood collection tubes on hormone measurements

Kristiina Ruotsalo

While it is known that hormone assay results can be affected by many factors, including medications, concurrent illness, as well as patient characteristics such as age and breed, the effects of the sample collection tubes themselves are frequently overlooked.

Collection of canine serum into serum separator tubes (SST) for progesterone analysis can result in spurious, time-dependent increases in total progesterone concentrations by the chemiluminescent assay used in most diagnostic laboratories. Similar increases in serum total thyroid hormone concentrations by the chemiluminescent technique have been reported with the use of SST tubes. Therefore, **we recommend that you collect samples for cortisol, thyroid, and progesterone hormone analysis into plain red-top plastic tubes**. Following sample clotting and centrifugation, transfer the serum to a clean plain red-top tube for transportation to the laboratory, with appropriate sample chilling or freezing as indicated in the AHL sample submission guidelines.

Please note that some hormones have unique collection requirements - **ACTH for example is very temperature sensitive** and requires collection of blood into **chilled EDTA tubes with immediate centrifugation**. The plasma should then be transferred into a plastic shipping tube, frozen, and sent with an ice pack. *AHL*

References

Hegstad-Davies RL. A review of sample handling considerations for reproductive and thyroid hormone measurement in serum or plasma. Theriogenology 2006;66:592-598.

Sunstrum J, Gartley C. Effects of blood collection tubes on concentrations of canine serum progesterone. Student poster presentation.

Immunohistochemistry tests available at the AHL

Josepha DeLay

Immunohistochemistry (IHC) is a useful procedure for the evaluation of both infectious disease and determination of the tissue of origin and, often, prognosis of various neoplasms. In IHC, antibodies specific for the infectious antigen or cell marker of interest are applied to formalin-fixed, paraffin-embedded tissue sections and the antigen-antibody interaction is visualized by a chromogenic indicator. **A major advantage of this procedure is the ability to demonstrate the specific antigen in association with microscopic lesions**, and IHC results are always interpreted with consideration of the histologic observations made on routine hematoxylin and eosin-stained tissue sections. IHC is not a 'stand-alone' test for the diagnosis of infectious disease, but is used in conjunction with histopathology and other ancillary tests that may be more sensitive.

Cell marker panels are designed by the pathologist interpreting a biopsy case and address the specific diagnostic requirements of the case. Because each neoplasm and the associated clinical situation are unique, we do not offer predetermined antibody panels.

For additional information or questions regarding IHC tests, please contact Dr. Josepha DeLay at jdelay@lsd.uoguelph.ca or ext. 54576. *AHL*

Infectious agents:
BVDV
Coronavirus:
- Bovine
- Feline (enteric and FIP)
- Canine
- Porcine (TGEV)

Canine distemper virus
Canine parvovirus 2
Chlamydophila spp
Feline panleukopenia virus
IBRV (*Bovine herpesvirus 1)*
Influenza A virus
Leptospira spp
Mycoplasma bovis
Neospora caninum
Porcine circovirus 2 (PCV-2)
PRRSV
TGEV
Toxoplasma gondii
West Nile virus (polyclonal / monoclonal)

Infectious agent panels:
Porcine respiratory panel: PRRSV, PCV-2, Influenza A virus
Bovine abortion panel: BVDV, IBRV, Leptospira spp

Cell markers:
Actin: smooth muscle actin, muscle actin
CD3
CD79a
CD18 (canine, feline)
CD31
CD61
CK117 (c-kit)
Chromogranin
Cytokeratins: pan CK (AE1/AE3), CK7, CK20, HMW CK
Desmin
Factor VIII-related antigen
GFAP
Hepatocyte paraffin antigen (HepPar 1)
Ki-67
Lambda light chains
Mast cell tryptase
Melan A
Neuron-specific enolase (NSE)
S100
Synaptophysin
Thyroglobulin
Thyroid transcription factor
Uroplakin
Vimentin

AHL Newsletter
December 2007 - Volume 11, Number 4

Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistant: **Ms. Helen Oliver**

The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

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.

New AHL submission forms - swine

Jim Fairles, Grant Maxie

Below is a draft of the new format for AHL submission forms, starting with swine as the prototype. The form will be one-page, with an NCR (no carbon required) second page to be retained in your clinic. We list the most commonly requested tests on the front of the page, with details on the components of panels, shipping instructions, etc. overleaf.

Our aims are several:

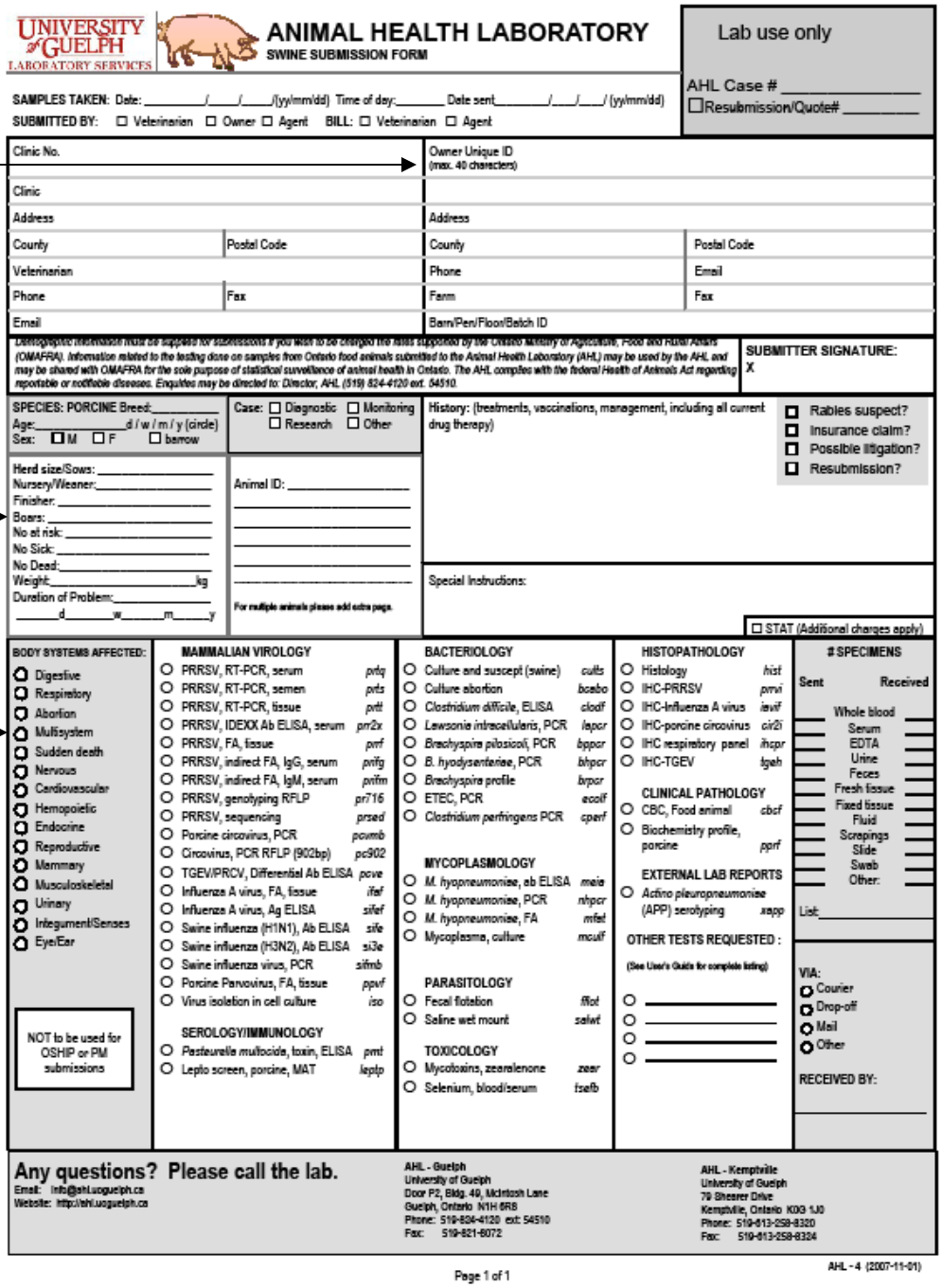
- Encourage compliance with completing demographic information by making this easier.
- Allowing syndromic surveillance by checking off a box for primary body system involved.
- Improving sample tracking through Sent and Received check-off boxes.

The latest draft of the swine form has been sent out to several clients, and we welcome your feedback. *AHL*

Owner unique ID - allows you up to 40 characters to specify how this will appear on your invoice - your client #, barn #, etc.

Production unit type

Body system affected - for syndromic surveillance



UNIVERSITY of GUELPH LABORATORY SERVICES

ANIMAL HEALTH LABORATORY
SWINE SUBMISSION FORM

Lab use only
AHL Case # _____
☐Resubmission/Quote# _____

SAMPLES TAKEN: Date: _____/____/____/(yy/mm/dd) Time of day:_____ Date sent_____/____/____/ (yy/mm/dd)
SUBMITTED BY: ☐ Veterinarian ☐ Owner ☐ Agent BILL: ☐ Veterinarian ☐ Agent

Clinic No.		Owner Unique ID (max. 40 characters)	
Clinic			
Address		Address	
County	Postal Code	County	Postal Code
Veterinarian		Phone	Email
Phone	Fax	Farm	Fax
Email		Barn/Pen/Floor/Batch ID	

Demographic information must be supplied for submissions if you wish to be charged the rates supported by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). Information related to the testing done on samples from Ontario food animals submitted to the Animal Health Laboratory (AHL) may be used by the AHL and may be shared with OMAFRA for the sole purpose of statistical surveillance of animal health in Ontario. The AHL complies with the federal Health of Animals Act regarding reportable or notifiable diseases. Enquiries may be directed to: Director, AHL (519) 824-4120 ext. 54510.

SUBMITTER SIGNATURE: X

SPECIES: PORCINE Breed:_____
Age:_____d / w / m / y (circle)
Sex: ☐M ☐F ☐ barrow

Case: ☐ Diagnostic ☐ Monitoring ☐ Research ☐ Other

History: (treatments, vaccinations, management, including all current drug therapy)

☐ Rabies suspect?
☐ Insurance claim?
☐ Possible litigation?
☐ Resubmission?

Herd size/Sows: _____
Nursery/Weaner: _____
Finisher: _____
Boars: _____
No at risk: _____
No Sick: _____
No Dead: _____
Weight: _____kg
Duration of Problem: _____
_____d _____w _____m _____y

Animal ID: _____
_____
_____
_____
_____
_____
For multiple animals please add extra page.

Special Instructions:

☐ STAT (Additional charges apply)

BODY SYSTEMS AFFECTED:
- ☐ Digestive
- ☐ Respiratory
- ☐ Abortion
- ☐ Multisystem
- ☐ Sudden death
- ☐ Nervous
- ☐ Cardiovascular
- ☐ Hemopoietic
- ☐ Endocrine
- ☐ Reproductive
- ☐ Mammary
- ☐ Musculoskeletal
- ☐ Urinary
- ☐ Integument/Senses
- ☐ Eye/Ear

NOT to be used for OSHIP or PM submissions

MAMMALIAN VIROLOGY
- ○ PRRSV, RT-PCR, serum *prtq*
- ○ PRRSV, RT-PCR, semen *prts*
- ○ PRRSV, RT-PCR, tissue *prtt*
- ○ PRRSV, IDEXX Ab ELISA, serum *prr2x*
- ○ PRRSV, FA, tissue *prrf*
- ○ PRRSV, indirect FA, IgG, serum *prifg*
- ○ PRRSV, indirect FA, IgM, serum *prifm*
- ○ PRRSV, genotyping RFLP *pr716*
- ○ PRRSV, sequencing *prsed*
- ○ Porcine circovirus, PCR *pcvmb*
- ○ Circovirus, PCR RFLP (902bp) *pc902*
- ○ TGEV/PRCV, Differential Ab ELISA *pcve*
- ○ Influenza A virus, FA, tissue *iftf*
- ○ Influenza A virus, Ag ELISA *sifef*
- ○ Swine influenza (H1N1), Ab ELISA *sife*
- ○ Swine influenza (H3N2), Ab ELISA *si3e*
- ○ Swine influenza virus, PCR *sifmb*
- ○ Porcine Parvovirus, FA, tissue *ppvf*
- ○ Virus isolation in cell culture *iso*

SEROLOGY/IMMUNOLOGY
- ○ *Pasteurella multocida*, toxin, ELISA *pmt*
- ○ Lepto screen, porcine, MAT *leptp*

BACTERIOLOGY
- ○ Culture and suscept (swine) *cults*
- ○ Culture abortion *bcabo*
- ○ *Clostridium difficile*, ELISA *cdif*
- ○ *Lawsonia intracellularis*, PCR *lapcr*
- ○ *Brachyspira pilosicoli*, PCR *bppcr*
- ○ *B. hyodysenteriae*, PCR *bhpcr*
- ○ *Brachyspira* profile *brpcr*
- ○ ETEC, PCR *ecolf*
- ○ *Clostridium perfringens* PCR *cperf*

MYCOPLASMOLOGY
- ○ *M. hyopneumoniae*, ab ELISA *mela*
- ○ *M. hyopneumoniae*, PCR *mhpcr*
- ○ *M. hyopneumoniae*, FA *mfat*
- ○ Mycoplasma, culture *mculf*

PARASITOLOGY
- ○ Fecal flotation *flot*
- ○ Saline wet mount *salwt*

TOXICOLOGY
- ○ Mycotoxins, zearalenone *zear*
- ○ Selenium, blood/serum *fselb*

HISTOPATHOLOGY
- ○ Histology *hist*
- ○ IHC-PRRSV *prrvi*
- ○ IHC-Influenza A virus *ievif*
- ○ IHC-porcine circovirus *circi*
- ○ IHC respiratory panel *ihcpr*
- ○ IHC-TGEV *tgeh*

CLINICAL PATHOLOGY
- ○ CBC, Food animal *cbcf*
- ○ Biochemistry profile, porcine *pprf*

EXTERNAL LAB REPORTS
- ○ *Actino pleuropneumoniae* (APP) serotyping *xapp*

OTHER TESTS REQUESTED :
(See User's Guide for complete listing)
- ○ _____
- ○ _____
- ○ _____
- ○ _____

 # SPECIMENS

Sent	Received	
		Whole blood
		Serum
		EDTA
		Urine
		Feces
		Fresh tissue
		Fixed tissue
		Fluid
		Scrapings
		Slide
		Swab
		Other:

List: _____

VIA:
- ○ Courier
- ○ Drop-off
- ○ Mail
- ○ Other

RECEIVED BY: _____

Any questions? Please call the lab.
Email: info@ahl.uoguelph.ca
Website: http://ahl.uoguelph.ca

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AHL Lab Reports

RUMINANTS

AHL offers BVDV real-time RT-PCR assay

Susy Carman, Beverly McEwen, Jim Fairles

The AHL will provide BVDV real-time RT-PCR testing starting Monday, December 3, 2007. **This PCR is designed to detect both BVDV type 1 and BVDV type 2 strains in one test.** The new real-time RT-PCR will replace our current gel-based RT-PCR for BVDV, and will be offered at the same price of **$28 per test**.

Our analytical validation shows this BVDV real-time RT-PCR to be a very sensitive test, with **sensitivity** of **5-10 CCID per mL**, similar to our current gel-based nested RT-PCR for BVDV. We have field-validated this new BVDV real-time RT-PCR in our laboratory by testing 198 clinical samples of serum, buffy coat, plasma, milk pellet, and ear notch in parallel with our current gel-based nested RT-PCR, and virus isolation. The results of this validation show the new BVDV real-time RT-PCR to have **sensitivity of 100%** (95% CI 76.8,100.0) and **specificity of 99.5%** (95% CI 97.0,100.0), to be specific when tested with a wide range of known bovine viral and bacterial pathogens, and to have **kappa value 0.96** (SE 0.037), implying almost perfect agreement with our other BVDV detection tests.

For this new real-time RT-PCR, the cut-off for positives will be Ct <36 (36 cycles). If the Ct is 36 to 40, the sample will be considered inconclusive and will be reported as “suspicious”. We can re-test these samples on request at an additional charge if no other samples are available. Negative samples will be reported as "undetected".

The new BVDV real-time RT-PCR does not distinguish between BVDV type 1 and BVDV type 2 strains. However the benefit of this new test is that it includes an internal positive RNA control to assess for the presence of PCR inhibitors, which can be present in clinical samples, making this new real-time RT-PCR better for initial screening. This test can be used to test ear notch samples, in which PCR inhibitors have been shown to be common. If PCR inhibition is recognized, the sample can be tested by another means.

There is ongoing discussion regarding the economic advantages of pooling serum or ear notches to detect persistently infected (PI) animals. **Since PI animals have high concentrations of BVDV in serum, we recommend that serum be used for herd screening**. We will continue to pool sera in groups of 5 when requested. If the pool is positive, then each sample in the pool will need to be tested individually by antigen detection ELISA. Larger pools can be made on request, but are awkward when the pool is positive. Final results are delayed and testing can eventually be more expensive, with the additional costs of either subsequent testing of smaller pools or when large numbers of samples need to be tested individually. The quantity of BVDV in ear notches of PI animals is low in comparison to blood. When ear notches were pooled in groups of 10 (1/10 dilution). a sensitive BVDV real-time RT-PCR missed 8% of known positives ear notches. When pooled in groups of 100, 67% of known positive ear notches were missed (1).

Please send ear notches individually. Please call to discuss your specific pooling questions and requirements.

We will subsequently type BVDV-positive samples as BVDV type 1 or BVDV type 2 at no extra charge using a second new BVDV real-time RT-PCR, specifically designed for typing. Typing will be batched, and results will follow in a few weeks.

Prosampler Ear Tissue sampling kits are available from Jenrik Ag, Minnesota at phone 877-251-2804 (strom@jenrik.com) for ease of collecting ear notches from large numbers of animals.

For more information on these new BVDV real-time RT- PCR assays, please contact Dr. Susy Carman at 519-824-4120 ext 54551, or scarman@lsd.uoguelph.ca *AHL*

Reference

1. Ridpath JF, et al. Evaluating stability, size requirements, viral load and pooling of ear notch samples in BVDV testing. 2006 International BVD Symposium, Denver, Colorado.

Bovine leukemia virus ELISA testing update *Davor Ojkic*

Because the IDEXX BLV ELISA has not been available for some time, the AHL has applied for and received a permit to import and use a European kit for detection of antibodies to *Bovine leukemia virus*. **We have received the kits from Europe and will be performing BLV testing as a 2-step procedure, screening and verification**. The screening test will be carried out on Mondays and cases with no reactive samples will be reported the same day. Any reactive samples will be tested with the verification procedure and will be reported on Tuesdays. Based on the manufacturer’s data, when compared to AGID the European BLV ELISA has sensitivity of 100.0% and specificity of 99.7%. The testing fee will remain at $7.00/test.

Continued on page 33

AVIAN/FUR-BEARING SPECIES

Genotyping of Canadian field strains of *Infectious bursal disease virus*

Davor Ojkic, Emily Martin, Janet Swinton, Brian Binnington, Marina Brash
(Abstract reprinted from Avian Pathology 2007;36:427-433.)

Infectious bursal disease virus (IBDV) was detected in 134 bursal samples that originated from flocks with conditions such as airsacculitis, tracheitis, pneumonia, septicemia, inclusion body hepatitis, coccidiosis, and/or a history of production problems without overt clinical signs.

Samples were from 7 Canadian provinces: Ontario, Quebec, Manitoba, British Columbia, Nova Scotia, Alberta, and Newfoundland and Labrador. Viral RNA was identified in bursae with moderate-to-severe and acute-to-chronic bursal damage. The ages of the flocks from which samples were collected ranged from 3 to 63 days. Following reverse transcriptase-polymerase chain reaction, the nucleotide sequence of the VP2 hypervariable region was determined and compared with sequences available in GenBank.

The most common Canadian IBDV field strains were North-American variant viruses. Forty-four viruses were highly related (97.5% to 100.0%) to the US IBDV strain NC171. Moreover, 16 field viruses whose VP2 sequences were 99.2% to 100% identical to the South African 05SA8 IBDV strain appeared closely related to the NC171 group. Delaware E-related field viruses, 98.3% to 100.0% identical to the prototype virus, were identified in 33 samples. Thirty-four Canadian IBDVs showed the highest identity, 94.2% to 98.3%, to US IBDV strain 586. Five samples contained vaccine-related viruses, while two field strains showed the best match to Del A (United States) and IBDV strains SP_04_02 (Spain). Very virulent IBDVs were not detected in Canada. *AHL*

Update on the 2007 Ontario Commercial Rabbit Enteritis/Food Safety Project

Patricia V. Turner, Marina Brash, Emily Martin, Durda Slavic, Scott Weese, Robert Wright, Brian Tapscott

The Ontario commercial rabbit industry provides an alternative meat source for Ontario consumers, sending approximately 200,000-250,000 animals to market annually. Since late 2005, the industry has experienced unexpectedly high mortality within herds, exceeding 40% in some cases. This has been primarily characterized by severe, persistent, watery diarrhea, dehydration, and wasting affecting all age groups. This has resulted in a shortage of fryers sent to abattoirs and a shortage of replacement does for breeding operations. The problem has affected well-managed operations of all sizes. Because of the narrow profit margin, producers are less likely to submit moribund animals on a regular basis for full diagnostic work-up, and it has been difficult to gain an adequate epidemiologic picture to assist the industry with herd management and to ensure public and food safety.

To address this issue, a collaborative study funded through the OMAFRA Food Safety Division was initiated in the spring of 2007. The study involves surveying the Ontario commercial rabbit industry for prevalence of infectious disease agents from clinically affected rabbits with diarrhea. **Two age groups, fryers and does (n = 40/age group), are being examined in the summer and winter of 2007 for prevalence of infectious disease agents.** Testing includes necropsy of clinically affected animals as well as histologic and microbiologic evaluations. In addition, the industry has been surveyed by questionnaire regarding on-farm hygiene and disposal practices.

Interim results of significant pathogenic agents isolated from the summer diagnostic surveillance are as follows:

- *E. coli* was found in fryers and does on all farms surveyed with enteropathogenic serotypes of *E. coli* identified in one operation;
- *Lawsonia intracellularis* was identified in less than 20% of the operations;
- *Clostridium spiroforme* was present in 30% of operations; and
- no animals were found to be positive for *Clostridium difficile*.

The results of the winter surveillance program may be different as environmental stressors tend to be more difficult to manage in cold, damp conditions. *AHL*

BLV ELISA, continued from page 32 Please read the fine print: This test is standardized against the international reference E4 according to the European Economic Community directive 88/406/EEC. However, the test kit has not been evaluated for specificity, sensitivity, reproducibility or quality control procedures by the Veterinary Biologics Section of CFIA. These limitations should be considered when interpreting the test results. Individual animal test results may vary and should be interpreted in light of other information such as history, clinical signs and other laboratory findings. *AHL*

SWINE

New swine submission form

Jim Fairles, Grant Maxie

Please see page 31 for a draft of this form. We welcome your comments!

HORSES

EHV-1 neurologic strains found in zebras

Susy Carman, Murray Hazlett, Jan Shapiro, Brian Binnington

Variation in a single amino acid in the DNA polymerase gene differentiates neuropathic strains of *Equid herpesvirus 1* (EHV-1) from non-neurologic strains. Using a real-time PCR test that is specific for EHV-1, and distinguishes potential EHV-1 neurologic strains from non-neurologic strains, **we have found archived zebra EHV-1 strains to be classed as EHV-1 neurologic strains.** These strains were originally isolated in cell culture and identified as EHV-1 strains using monoclonal antibodies.

The first strain was isolated in 2002 from the lung of a 4-month-old male zebra with severe necrotizing bronchiolitis, syncytial cell formation, intranuclear inclusions and vasculitis in the lung. In addition there was EHV-1 monoclonal antibody-specific IHC staining in bronchiolar epithelium and adjacent alveolar pneumocytes. Single cell necrosis was identified in the liver.

The second strain was isolated in 2007 from pooled tissue, including brain, of an 8-month-old female zebra with erosive to ulcerative dermatitis, stomatitis, gingivitis, gastritis, enterocolitis, acute severe multifocal necrotic hepatitis, splenitis, and pneumonia associated with acute vasculitis. Multinucleated cells and intranuclear inclusions were present in the lung. The lung was positive in a direct FA test using polyclonal antisera specific for EHV-1/4.

These findings are important to the horse industry. **Racetracks and fairs have petting zoos that may include zebras, which could be a source of infection for horses of neurologic strains of EHV-1.**

References

Allen GP, Development of a real-time polymerase chain reaction assay for rapid diagnosis of neuropathogenic strains of equine herpsevirus-1. J Vet Diagn Invest 2007;19:69-72.

Nugent J, et al. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. J Virol 2006;80:4047-4060.

Shapiro J, Binnington B. Four interesting equine cases from eastern Ontario. AHL Newsletter 2007;11:26-27.

Sudden unexpected death in equids

Jan Shapiro, Beverly McEwen, Brian Binnington

Between January 1, 2000 and April 30, 2007, **70 horses and 1 donkey**, representing 11.2% (71/365) of the total equine necropsy cases, were submitted to the Animal Health Laboratory in Guelph and Kemptville. All had a history of having been found dead in the stall or on pasture, or having dropped dead during exercise with no prior clinical signs.

The gastrointestinal system was the most common body system involved in sudden death, involving 23 % of cases. Cardiovascular system lesions were found in 13 % of cases, and were overrepresented in recently foaled mares. In only 7% of cases was infection the primary cause of death. In 8% of cases, there were no significant gross or histopathologic findings.

The diagnoses listed in the text below were single cases, unless otherwise indicated.

Diagnoses in foals **1 day to 1 month old** were small colon impaction and rupture with septic peritonitis, Tyzzer's disease (2 cases), ruptured bladder, acute clostridial enteritis, and hyperplastic goiter.

Diagnoses in foals **1 month to 6 months old** were perforated duodenal ulcer, acute mesenteric torsion, ileocecal intussusception with perforation and peritonitis, Tyzzer's disease, fractured occipital bone, external umbilical hernia, and no significant lesions.

Diagnoses in horses between **6 months and 2 years** of age were fractured proximal cervical vertebra, myocardial infarcts and vegetative valvular endocarditis, Japanese yew toxicity (donkey), and acute torsion of the colon.

Sudden unexpected death in equids - continued from p 34

All of the horses that died during exercise were adult race horses - 4 Thoroughbreds (TB) and 5 Standardbreds (SB). Diagnoses included aortic rupture (TB), exercise-induced pulmonary hemorrhage (TB), idiopathic acute pulmonary edema and hemorrhage (1 TB, 1 SB), idiopathic multisystemic acute hemorrhage with terminal septicemia, acute hemoperitoneum (SB), myocarditis (SB) and no significant lesions (1 TB, 1 SB).

The largest group of horses submitted was **adults over 2 years of age** found dead in their stall or on pasture. In Thoroughbreds, diagnoses included gastric dilation associated with gastritis, acute perforation of the colon, large colon torsion with infarction, cecal torsion, cecal rupture with acute peritonitis, acute pelvic fracture with hemoperitoneum, myocardial hemorrhage, presumptive acute anaphylaxis, Japanese yew toxicity, and hemoperitoneum due to ruptured ovarian artery. Diagnoses in Standardbreds were diaphragmatic hernia, acute gastric rupture (3 cases), mesenteric torsion, presumptive ileus and intestinal dilation, multifocal myocarditis (2 cases), idiopathic acute pulmonary edema, acute bloat, and no significant lesions (3 cases). Diagnoses in other breeds of horses were torsion of the large intestine, severe acute neck trauma, mild meningitis, and no significant lesions (3 cases),

Mares found dead within 1 week of foaling bled internally due to a ruptured uterine artery. However, 1 had intragastric hemorrhage associated with multiple deep ulcers of the pars esophagea. *AHL*

Encephalitis due to *Sarcocystis neurona* in a Standardbred horse

Murray Hazlett, Josepha DeLay, Bernard Ferguson

A 7-year-old, previously healthy, Standardbred mare was found in distress, disoriented, able to stand but walking in circles and stumbling. The mare had a head tilt to the right, a dropped right ear and lower right lip, a fixed gaze and ataxia, indicative of involvement of several cranial nerves. Despite supportive treatment, the mare did not improve, requiring assistance to rise, and becoming a hazard to handlers. She was euthanized 10 days after the initial clinical signs appeared and submitted for necropsy.

Although no significant gross lesions were seen, microscopically there was severe nonsuppurative encephalitis with focal glial nodules in midbrain and medulla. A single structure resembling a protozoal cyst was seen in association with inflammatory foci in H&E sections. Immunohistochemistry (IHC) for *Sarcocystis neurona* at Prairie Diagnostic Services (PDS) confirmed sarcocystosis (Figure 1).

Since 1999, the AHL has had 12 confirmed cases of *S. neurona* encephalitis and/or myelitis in horses (confirmed with IHC). Time of onset from clinical signs to necropsy averaged 25 days (4-120). Despite formerly being known as equine protozoal myelitis, 6 of 8 cases where both brain and cord were examined had lesions in both areas, most often in brain stem, and **now EPM refers to equine protozoal myelo*encephalitis*.** Two cases had only brain examined because of the predominant clinical signs – interestingly both of these horses were reported as circling, with a head tilt, drooping lip, and ataxia.

Most cases occurred in the time period between June and December (Figure 2), with no cases in mid-winter, perhaps because of reduced activity of the definitive host (the Virginia opossum). **With the increasingly mild winters we are expecting, opossum populations will likely increase, and we can speculate that there may be an associated increase in cases of equine encephalomyelitis associated with *S. neurona*.** It is noteworthy that domestic cats may serve as intermediate hosts, as do skunks and raccoons, with the carcasses being scavenged by the opossum. Horses are considered an aberrant or dead-end host. *AHL*

Reference

Stanek JF, et al. Epidemiology of *Sarcocystis neurona* infections in domestic cats (*Felis domesticus*) and its association with equine protozoal myeloencephalitis (EPM) case farms and feral cats from a mobile spay and neuter clinic. Vet Parasitol. 2003;117:239-49.

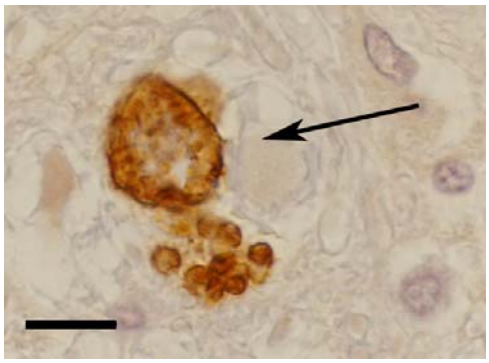

Figure 1. IHC showing *S. neurona* (arrow) in brain. Bar = 10um**.**

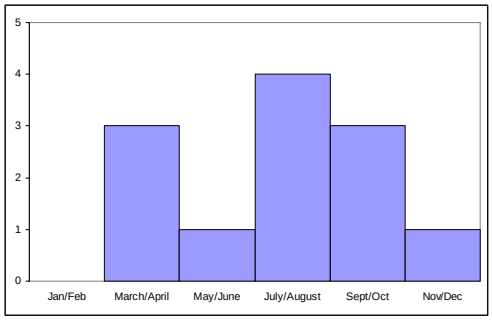

Figure 2. AHL sarcocystosis diagnoses by month

COMPANION ANIMALS

Blastomycosis in a cat

Brian Binnington, Jan Shapiro, Murray Hazlett

A 9-year-old cat was treated for 2 weeks for a suspected submandibular abscess with antibiotic (Clavamox) and anti-inflammatory (Ketoprofen) drugs without response. A fine-needle aspirate of the mass was consistent with pyogranulomatous inflammation and contained numerous poorly stained yeast-like bodies suggestive of *Blastomyces*. This cat had access to the outdoors along the Rideau River in eastern Ontario. The cat was subsequently euthanized and submitted to AHL-Kemptville for necropsy.

A mass extended from the margin of the left lower lip to the angle of the mandible, caudally to the mid-lateral and ventral neck region and dorsally to involve the subcutis and the muscles at the base of the left ear and the left side of the face. On cut surface, the solid mass was variegated tan, white and brown with a firm-to-hard consistency with some softer to gelatinous regions. The mass at the angle of the mandible encompassed salivary gland, and the cervical lymph nodes were mildly enlarged. There were 8 to12 solid round tan-pink 2 to 4 mm nodules in the lungs. Sections of skin, subcutaneous tissues, muscle, salivary glands, lymph node and lung had multifocal and coalescing areas of intense pyogranulomatous inflammation characterized by the accumulation of epithelioid macrophages and neutrophils with areas of necrosis and cellular debris. There were areas of cutaneous ulceration, with necrosis and intense pyogranulomatous inflammation with fibroplasia in the deep dermis and subcutaneous tissues. Numerous round-to-ovoid yeast were present in the exudates and in the cytoplasm of macrophages. The yeasts were non-encapsulated, with a prominent double-contoured cell wall, central granular protoplasm and variable sizes from approximately 10 to 20 µm diameter with a few 30 µm diameter organisms. Silver staining clearly demonstrated the argyrophilic cell wall and broad-based budding of the yeast (Figure 1)

The morphology of the yeast was compatible with *Blastomyces dermatitidis*, a dimorphic fungus with a mycelial form that produces infectious spores, and a yeast form in tissues at body temperature that reproduces asexually by budding. The reservoir for *B. dermatitidis* is thought to be soil, in which it grows as the infectious mycelial form. Environmental growth appears to require sandy, acid soil in proximity to water. Proximity to waterways is considered to be a risk factor, although cases of blastomycosis have occurred in strictly indoor dogs and cats.

Exposure in most animal and human cases is considered to be inhalation of the spores from the mycelial form in the environment. The yeast form in tissues is considered to be of low infectivity, and it is not transmitted from animal to people or people to people through aerosols. Penetrating wounds by objects contaminated by the organism have been rarely reported to produce infections in people. The outcome of infections is likely affected by the immune status and immunosuppression by a variety of factors results in more fulminant infections. *AHL*

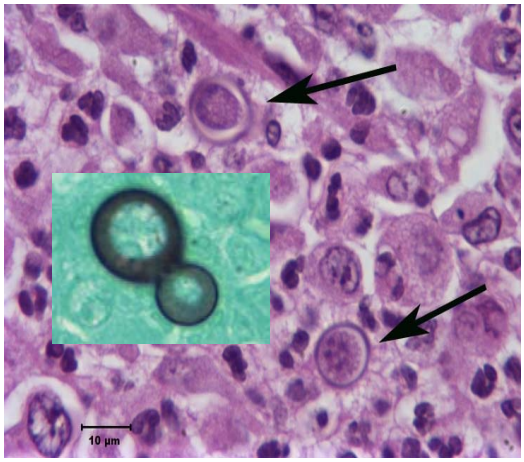

Figure 1. *Blastomyces dermatitidis* (arrows), broad-based budding with silver stain (inset).

Canine sebaceous adenitis (SA) testing at the AHL *Josepha DeLay*

Histopathology of biopsies for detection of sebaceous adenitis is available through the AHL for registration in the Orthopedic Foundation for Animals (OFA) SA database. Dogs are classified according to combined histologic lesions and clinical findings. Submissions must include the biopsy samples, completed and signed SA database application form, and separate OFA payment. Regular AHL histopathology fees are billed to the referring clinic. The AHL reports results to both the referring veterinarian and to OFA. Application forms and specific biopsy instructions are available on the OFA website: http://www.offa.org/index.html **Please contact the AHL if you have any questions about SA testing for your clients.**

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