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Animal Health Laboratory
University of Guelph
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AHL Newsletter

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In this issue:

AHL Virology Laboratory Highlights

- Mammalian and avian virology laboratory recently "re-united" after moving into the new Pathobiology-Animal Health Laboratory building
- 21 technical staff
- 2 veterinarians specializing in virology - Dr. Susy Carman, Mammalian Virology; Dr. Davor Ojkic, Avian Virology and Immunology
- Both classical and molecular virology diagnostic testing procedures are available:
  - Virus isolation in cell culture and SPF eggs
  - Virus neutralization and hemagglutination-inhibition antibody detection assays
  - Fluorescent antibody testing
  - Antigen and antibody ELISAs
  - Real time PCR
  - Gene sequencing/genotyping

AHL Virology group:

Back row: Keith Harron, Bhaju Tamot, Christian Sandrock, Li Ge.

Second row: Hongwen Fu, Mioara Antochi, Dr. Davor Ojkic, Gaye Smith, Joanna Sawicki, Christine Mason.

Third row: Dr. Susy Carman, Anna Marom, Lynn Henry, Jonathan Chang, Rebecca Marshall.

Front row: Sarah Hoyland, Jane Coventry, Elizabeth Hillyer, Angelique Eberle.

Missing from the photo: Thelma Martinez, Liliana Perez Toledo, Linda Little, Grant Perry, Sophia Lim.

Note to companion animal and equine practitioners re submissions for AHL histopathology

Please be aware that AHL histopathology charges were adjusted in May 2011 to reflect the number of biopsies or tissues included with each companion animal or equine histopathology submission.

- **Cases with 1-2 biopsies or tissues are charged $69; cases with 3 or more tissues or biopsies are charged $95.**
- **Exceptions** to this rule include multiple (4-6) cutaneous punch biopsies (6 mm or less), gastrointestinal endoscopic biopsies, and Tru-cut or needle biopsies, for which the $69 fee applies due to the small size of these samples.
- For excisional biopsies on which **margin evaluation** is requested, a $30 charge applies in addition to the routine biopsy fee.

Please contact the AHL if you have questions regarding individual submissions.

AHL peer-reviewed publications, 2011

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Ganeshapillai J, Boncheff AG, **Slavic D**, MacInnes JI, Monteiro MA. The lipopolysaccharide core of *Actinobacillus suis* and its relationship to those of *Actinobacillus pleuropneumoniae*. Biochem Cell Biol 2011;89:351-358.

Hazlett MJ, Kircanski J, **Slavic D,** Prescott JF. Beta 2 toxigenic *Clostridium perfringens* type A colitis in a three-day-old foal. J Vet Diagn Invest 2011;23:373-376.

Herdt TH, **Hoff B**. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet Clin Food An 2011;27:255-283.

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O'Sullivan T, Friendship R, Blackwell T, Pearl D, **McEwen B, Carman S, Slavic D, DeLay J,** Dewey C. Microbiological identification and analysis of swine tonsils collected from carcasses at slaughter. Can J Vet Res 2011;75:106-111.

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Rebelo AR, Parker L, Cai HY. Use of high-resolution melting curve analysis to identify *Mycoplasma* species commonly isolated from ruminant, avian, and canine samples. J Vet Diagn Invest. 2011;23:932-6. Epub 2011 Aug 19.

Saam DE, Liptak JM, **Stalker MJ,** Chun R. Predictors of outcome in dogs treated with adjuvant carboplatin for appendicular osteosarcoma: 65 cases (1996-2006) J Am Vet Med Assoc 2011;238:195-206.

Slavic D, Boerlin P, Fabri M, Klotins KC, **Zoethout JK, Weir PE, Bateman D**. Antimicrobial susceptibility of *Clostridium perfringens* isolates of bovine, chicken, porcine and turkey origin from Ontario. Can J Vet Res 2011;75:89-97.

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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.

Selected zoonotic pathogens and diseases identified at the AHL, 2011

Beverly McEwen, Durda Slavic, Davor Ojkic, Josepha DeLay, Hugh Cai, Margaret Stalker, Murray Hazlett, Marina Brash

Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens in over 1,000 cases annually (Tables 1 and 2). These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates. Monitoring programs are not included.

The zoonotic pathogens most frequently identified at the AHL since 1999 are *Leptospira* spp., *Salmonella* sp., *Streptococcus* sp., and *Cryptosporidium* sp. Occupational exposure to pigs and horses is a risk factor for *S. suis* and *S. zooepidemicus* infections. This is the first year we have included methicillin-resistant *S. pseudintermedius*.

The continued increase in cases of *Coxiella burnetii* and *Chlamydophila sp*. is due to the increased surveillance for these pathogens in the small ruminant abortion project. Veterinarians must report suspected cases of *Chlamydophila psittaci* to the Ontario Ministry of Health.

Under "Other", EEEV infection in ring-necked pheasants from an upland game hunting reserve and WNV infection in farmed ducks are reported for the first time in Ontario.

Prior to 2008, the numbers of isolates were tabulated, however, due to the increasing number of tests for selected pathogens, the number of cases will now be documented. For data prior to 2008, please refer to previous editions of the AHL Newsletter. *AHL*

Table 1. Cases with selected zoonotic pathogens isolated and/or identified at the AHL, 2011 (includes out-of-province testing)

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	**2011**	2010	2009	2008
Blastomyces dermatitidis								9	1		**10**	5	10	
Bordetella bronchiseptica		28	2					6		7	**43**	54	60	52
Borrelia burgdorferi (Lyme disease)								1			**1**			
Campylobacter coli/ jejuni/ fetus subsp. *fetus*				12							**12**	24	14	14
Chlamydophila sp.				15	21					3	**39**	58	29	10
Clostridium difficile	2	14	8			14		1		1	**40**	31	24	25
Coxiella burnetii (Q fever)	1			48	50						**99**	115	9	15
Cryptosporidium sp.	128	4		3	8					4	**147**	157	128	144
Eastern equine encephalitis virus			4							1	**5**	12	11	12
Giardia sp.	13			1				15	2		**31**	60	55	56
Listeria monocytogenes	8	1		4	5						**18**	19	18	14
Methicillin-resistant *Staphylococcus aureus* (MRSA)			11						2		**13**	74	36	51
Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)			3					63	4	4	**74**			
Mycobacterium tuberculosis											**0**	0	0	1
Rabies											**0**	3	8	4
Salmonella sp.	56	61	8	13	1	78	10	2	1	26	**256**	256	281	322
Streptobacillus moniliformis (rat bite fever)											**0**	0	0	1
Streptococcus suis	1	101		2	2						**106**	110	120	158
Streptococcus equisimilis	1	29	18	2				5		4	**59**	48	43	76
Streptococcus zooepidemicus	6	2	130					2	1	8	**149**	152	117	108
Toxoplasma sp.				13	10				1		**24**	22	19	8
West Nile virus			10							24	**34**	7	6	70
Yersinia enterocolitica		1									**1**	2	0	6

Table 2. *Leptospira spp*. seropositive cases identified at AHL, 2011, microscopic agglutination test (MAT) (includes out-of-province test-

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Other & not specified
L. autumnalis	3	0	5	33	1
L. bratislava	5	6	8	9	0
L .canicola	2	0	1	8	0
L. grippotyphosa	6	1	2	27	1
L. hardjo	2	0	0	0	0
L. icterohaemorrhagiae	10	2	4	7	0
L. pomona	25	3	6	25	1

AHL Lab Reports

RUMINANTS

Bovine pneumonia due to *Bibersteinia (Pasteurella) trehalosi*

Jan Shapiro, Durda Slavic, Beverly McEwen

In 2007, based on genotypic and phenotypic testing, *Pasteurella trehalosi,* originally described as **biotype T of *Pasteurella haemolytica*,** was transferred to the genus *Bibersteinia.* **Effective January 2012, AHL bacteriology reports of this organism also use the new name *Bibersteinia trehalosi*.**

B. trehalosi is an important pathogen of sheep, causing acute systemic infection and death in growing lambs, but it has also been isolated from other ruminants including cattle, goats and bison. A review of bovine cases submitted to the AHL in Guelph and Kemptville between 2007 and 2011 showed that, ***B. trehalosi* was cultured from the lungs of 12 cattle submitted for necropsy and/or histology that were diagnosed with primary pneumonia, and from 6 calves diagnosed with septicemia.**

Of the 12 pneumonia cases, 5 were dairy cattle >1 year of age (range 14 mo-13 yr), 6 were dairy cattle <1 yr of age (range 2 wk -7 mo), and 1 was a 7-mo-old beef calf. *B. trehalosi* was isolated from the lungs in pure culture in 2 cases and in mixed culture with other bacteria such as *Pasteurella multocida, Arcanobacterium pyogenes, Mannheimia haemolytica, Histophilus somni* and *E. coli* in 9 cases. Eight pneumonia cases were also tested for other respiratory pathogens - 1 or more *Mycoplasma spp* were cultured in 4 cases, and *Ureaplasma diversum* in 4 cases. No viral pathogens were detected in the 4 cases tested. The histological characterization for the lung lesions was fibrinopurulent bronchopneumonia (6 cases), necrotic or fibrinonecrotic bronchopneumonia (3 cases), fibrinous bronchopneumonia (2 cases), or bronchointerstitial pneumonia (1 case).

Of the 6 septicemia cases, 3 were dairy cattle under 7 mo of age and 3 were beef cattle under 6 mo of age. In 2 cases, *B. trehalosi* was isolated in pure culture, and in 3 cases it was in mixed culture with *E. coli, Mannheimia haemolytica*, *Klebsiella oxytoca* or *Salmonella* spp. In 1 case, BVDV was also cultured.

Analysis of antibiograms of *B. trehalosi* showed **highest resistance to erythromycin, 72% (13/18), followed by resistance to sulfonamides and tetracycline, 27% (5/18). Low resistance (5%) was detected for tilmicosin and tulathromycin (1/18). No resistance was detected for ceftiofur, spectinomycin, or florfenicol.** *AHL*

Bovine abortion diagnoses, 2006 - 2012

Maria Spinato, Beverly McEwen, Andrew Brooks, Josepha DeLay, Murray Hazlett, Jan Shapiro, Margaret Stalker, Tony van Dreumel, Susy Carman, Durda Slavic, Hugh Cai

An examination of bovine abortion pathology cases submitted to the AHL over the past 6 years indicates some interesting trends, summarized in Table 1.

Abortions caused by *Neospora* sp., various bacterial and mycotic species, and BVDV are diagnosed in low numbers each year. A spike in abortions caused by *Ureaplasma diversum* was noted in 2010/11. Approximately 10% of fetuses submitted for examination in 2007/08 and 2011/12 were diagnosed with congenital anomalies. In 2011/12, the most common anomalies were cardiac defects (5). Three cases were associated with various developmental brain disorders, and 2 involved musculoskeletal malformations. One case each of twin fetuses and brachyspina syndrome of Holstein cattle were also diagnosed.

The number of infectious bovine rhinotracheitis virus (IBRV/ (*Bovine herpesvirus 1*) positive cases doubled within the first 8 months of the 2011/12 reporting year, compared to the previous 12-month period. Only 2 of these cases were associated with either failure to vaccinate (1 herd), or unknown vaccination status (1 herd). All of the remaining 5 herds (multiple submissions for 1 herd) reported the use of IBRV modified live virus vaccines. While most producers indicated that they had followed the manufacturer's recommended guidelines for dosing, one submitter believed that they were using a killed virus vaccine. **A common feature of these IBRV cases associated with MLV vaccines was a relatively high number of abortions occurring in a short time interval**, ranging from 3-17 animals ('abortion storm'). Mainly heifer dams were affected in 2 herds.

The probability of obtaining an etiologic diagnosis in cases of bovine abortion is greatly increased if the entire fetus and placenta are submitted for examination. In cases where it is more practical to submit fresh and formalin-fixed tissues, veterinarians are advised to consult the AHL User's Guide for details. *AHL*

Table 1. Pathology diagnoses, bovine abortion cases, May 2006 - Jan 2012 (*multiple diagnoses possible)

Diagnosis	2006/07	2007/08	2008/09	2009/10	2010/11	2011/12 (Jan 2012)
Total abortions submitted	**150**	**127**	**111**	**116**	**131**	**108**
No lesions/etiology identified	68	39	54	45	49	14
Placentitis, etiology not identified	26	16	22	22	29	33
Neospora sp.	17	6	8	8	9	9
Bacterial (misc or not specified)	12	24	9	18	10	18
BoHV-1/IBRV	0	1	5	2	4	8
Arcanobacterium pyogenes	7	7	6	9	10	6
Mycotic	3	8	1	1	4	2
BVDV	4	5	0	1	1	2
Bacillus licheniformis	5	2	1	0	2	0
Listeria monocytogenes	1	3	0	3	0	1
Ureaplasma diversum	4	5	3	2	10	0
Coxiella burnetii	0	0	0	0	0	1
Anomaly	2	11	3	1	1	12
Leptospira sp.	2	0	2	1	2	1
Mycoplasma bovigenitalium	0	1	0	1	1	2
Other *Mycoplasma* sp.	0	0	0	0	1	0
Other (trauma)	0	1	1	0	0	0
Goiter	1	0	0	2	2	0
Total diagnoses *	**155**	**129**	**115**	**116**	**135**	**109**

Polyserositis (alpaca fever) caused by *Streptococcus equi* subsp. *zooepidemicus* in a llama

Murray Hazlett, John Las, Robert Perry, Durda Slavic

A 1.5-year-old male llama was examined as it was in lateral recumbency and reluctant to rise. Clinically the animal had muffled heart sounds, lung crackles with an increased respiratory effort and rate, and a fever of 41.2°C. Rumen contractions were weak and there was mild bloat. It was treated with florfenicol and flunixin. Two days later the llama was treated medically for bloat, however the animal became weak, recumbent and died about 36 hours later. The animal was housed with a mixture of about 25 alpacas and llamas. Three horses were also in the barn.

Necropsy revealed acute severe diffuse fibrinous pleuritis with diffuse pulmonary congestion. About 1-2 cm of fibrin with no fibrosis was present bilaterally over the surface of both visceral and parietal pleura. The epicardium was also covered with about 1 cm of fibrin, and a 1-2 cm layer of fibrin was present over the ventral and right side of the stomachs, extending along the ventral abdominal wall to include the pelvic inlet. Bacterial culture of lung recovered a pure culture of *Streptococcus equi* subsp *zooepidemicus*.

Polyserositis has been previously associated with *S. equi* subsp *zooepidemicus* in llamas and alpacas, and herd outbreaks of polyserositis due to this organism have been described. It may be carried in clinically normal horses in the nasopharynx. ***S. equi* subsp. *zooepidemicus* is the cause of "alpaca fever" in Peru, with the acute polyserositis form usually seen in younger animals, and chronic abscessation seen in older animals.** *AHL*

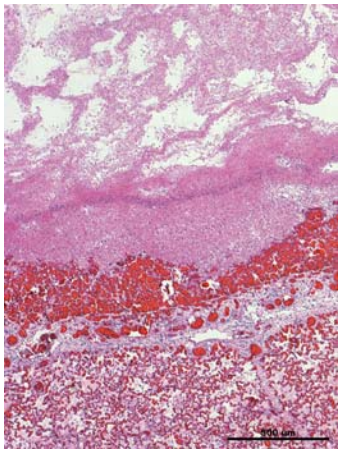

Figure 1: Fibrin with enmeshed bacteria overlying the pleural surface of the lung.

SWINE

Preliminary comparison of Oxoid and IDEXX *Mycoplasma hyopneumoniae* ELISAs

Hugh Cai, Hamid Haghighi, Beverly McEwen, Jim Fairles

Recently the AHL evaluated *M. hyopneumoniae* ELISA kits manufactured by Oxoid (lot # 987572) and IDEXX (lot #06733-HG564) with a limited number of field samples, consisting of 55 swine sera selected from 10 different submissions with bias to include more positive and suspicious samples. All samples were tested with the two ELISAs in parallel. To determine the repeatability of the assays, 30 samples were tested 3 times with both kits on 3 consecutive days. Although there was no significant difference ($p = 0.65$) between the number of positive samples identified by the Oxoid and IDEXX ELISAs, the Oxoid ELISA identified significantly more suspicious samples ($p<0.01$) (Table 1). Without a gold standard, we cannot determine if the discrepancy in results was caused by better sensitivity or poorer specificity of the Oxoid ELISA. The kappa value for repetitive tests was 0.48 for the Oxoid ELISA and 0.86 for the IDEXX ELISA, indicating that **the IDEXX ELISA had much better repeatability.**

Although the AHL continues to offer both Oxoid and IDEXX *M. hyopneumoniae* ELISAs, **we recommend using the IDEXX *M. hyopneumoniae* ELISA as the primary test.** *AHL*

Table 1. Comparison of Oxoid and IDEXX *Mycoplasma hyopneumoniae* ELISAs with selected field serum samples

***Mycoplasma hyopneumoniae* ELISA**	**Positive**	**Suspicious**	**Negative**	**Total**
Oxoid	15	34	6	55
IDEXX	12	4	39	55

*The overall agreement (Kappa) among all Oxoid and IDEXX results was 0.39.

AHL now offers IDEXX PRRSV X3 antibody ELISA testing for swine oral fluids

Susy Carman, Mioara Antochi, Joanna Sawicki

The Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) has developed methods to adapt the IDEXX PRRSV X3 antibody serum ELISA for the identification of PRRSV IgG antibody in swine oral fluids. The ISU-VDL has shown that **oral fluids can be used to assess both maternal antibody and antibody that follows PRRSV infection**, with sensitivity of 95.0% and specificity of 100% at S/P ratios ≥ 0.4.

The AHL regularly tests oral fluids for PRRSV using PCR. Recently, we participated in the ISU-VDL sponsored IDEXX PRRSV X3 antibody ELISA ring test for swine oral fluids, using the test protocol developed by Dr. Jeff Zimmerman, and we now offer this test.

- Oral fluids are collected with cotton ropes from animals of all ages.
- The oral fluids must be harvested from the rope into a 5 mL screw top container before submission on ice packs.
- Test modifications require that oral fluids be run separately from serum, with an overnight incubation, making this a 2-day test.
- Testing for oral fluids begins on Tues, Wed, Thurs, with results reported the next day.
- The results for the oral fluid ELISA are interpreted the same as for the serum test, with **S/P ratios ≥ 0.4 considered positive.**
- The fee is $12.75 per test.

For more information please contact Dr. Susy Carman 519-824-4120 ext 54551 or scarman@uoguelph.ca . *AHL*

Reference

Kittawornrat S, et al. Detection of *Porcine reproductive and respiratory syndrome virus* (PRRSV) antibodies in oral fluid specimens using a commercial PRRSV serum antibody ELISA. J Vet Diagn Invest 2012 (in press).

Porcine reproductive and respiratory syndrome virus-associated vasculitis in nursing piglets

Josepha DeLay, Margaret Stalker, Susy Carman, Al Scorgie

A 300-sow farrow-to-finish herd experienced an increase in perinatal loss beginning in early December 2011. Initially, all 10 periparturient sows in one all-in/ all-out farrowing room farrowed prematurely, with a high stillbirth rate and weak neonates. In addition, sows in the gestation barn were anorexic and pyrexic, and clinical signs gradually progressed throughout the barn. Over the following 4 weeks, 24 sows in mid- to late gestation farrowed prematurely or aborted, and 4 sows died. ***Porcine reproductive and respiratory syndrome virus* (PRRSV) infection was suspected as the cause of systemic illness in sows and fetal infection**.

PRRSV was identified by RT-PCR in the serum from 1 of 7 live piglets tested, but not from the pooled tissues of 3 liveborn piglets submitted to the AHL for diagnostic workup. **PRRSV ORF5 sequencing identified a new PRRSV field strain for this herd, with sequence predicted RFLP type 1-18-4.** The pooled tissues of 2 of the 3 live piglets tested were positive for *Porcine circovirus 2* (PCV-2) on PCR. Fluorescent antibody tests for PPV were negative. No other pathogens were identified in this group. Multifocal hepatic necrosis was identified histologically in 1 stillborn fetus from the same litter as the PRRSV-positive liveborn pig, however no etiologic agent was identified in this fetus.

Abortions and clinical illness in sows decreased over the following month. However a subsequent increase in mortality was identified in nursing piglets 2 weeks after the abortions began. Three piglets ranging from 1-3 kg body weight were submitted to the AHL for necropsy. Suppurative polyarthritis was evident in 2 piglets, and multifocal pinpoint renal cortical hemorrhages were present in 1 piglet with polyarthritis and in the third piglet from the group. Histologically, the piglet with both joint and renal lesions had multiple foci of vasculitis (primarily arteritis), with thrombosis in lung, kidney, and brain. **Vascular lesions were associated with abundant PRRSV antigen in vessel walls, as demonstrated by immunohistochemistry** (IHC; Figure 1). In addition, all piglets had mild to moderate lymphohistiocytic interstitial pneumonia also associated with PRRSV antigen. IHC tests for PCV-2 were negative. PRRSV RT-PCR tests on pooled lung and tonsil samples tested from each individual piglet were positive for field-strain PRRSV virus, with a similar PRRSV ORF 5 sequence predicted RFLP type 1-18-4, with 99.8% sequence homology to the strain detected in the weak liveborn piglet and submitted 2 weeks before. Histologic lesions of polyserositis were evident in 2 piglets, and *E. coli* was isolated from multiple sites in 1 of these animals.

Abortion can be associated with both PRRSV and PCV-2. Vasculitis (including arteritis) is rare in association with PRRSV infection. It has been described in umbilical cord of fetuses aborted due to PRRSV infection, and in brain and other tissues of fetuses and liveborn piglets both naturally and experimentally infected with PRRSV *in utero*. In pigs, vasculitis and/or vasculopathy can be caused by other viruses, including PCV-2, *Bovine viral diarrhea virus*, *Classical swine fever virus* (CSFV), *African swine fever virus* (ASFV), *Ovine herpesvirus 2*, and *Suid herpesvirus 1* (SuHV-1, Pseudorabies virus), as well as by various bacteria and vitamin E /selenium deficiency. Because of evidence of vasculitis in 1 nursing piglet in the herd, tissues from all 3 animals in the submitted group were forwarded to CFIA for testing for CSFV and ASFV. Tissues from the first group were also tested for SuHV-1 due to the unusual hepatic necrosis in 1 stillborn fetus. PCR test results for CSFV, ASFV, and SuHV-1 were negative.

Regardless of the presence of suspicious lesions, **this case highlights the importance of including foreign animal diseases among the differential diagnoses in clinical situations in swine herds where reproductive loss and systemic illness are ongoing, and for which a more common etiologic agent cannot be identified**. The cause of early farrowings, stillbirths, and weak neonates in this herd can be attributed to PRRSV and PCV-2 infection, with sow infection with PRRSV, based on lesions and documented PRRSV and PCV-2 infection in live-born littermates. *AHL*

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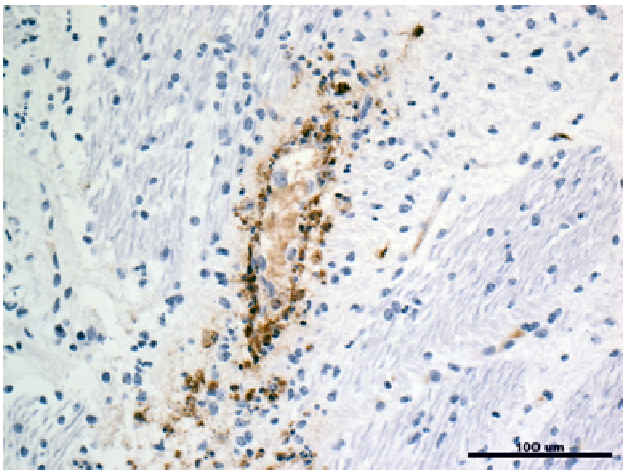

Figure 1. Piglet brain: PRRSV antigen (brown staining) associated with histologic evidence of vasculitis. IHC, Nova red chromogen.

AVIAN/FUR/EXOTIC SPECIES

West Nile virus meningoencephalitis in a wild Eastern grey squirrel (*Sciurus carolinensis*)

Jan Shapiro, Doug Campbell

On September 27, 2011, a 176 g female juvenile black-phase wild Eastern grey squirrel, estimated to be 5-8 weeks old, was submitted to the AHL-Kemptville for necropsy. The squirrel was one of 4 in a litter admitted to a wildlife sanctuary just south of Ottawa a few days previously. In the preceding 11 days, 11 of 20 squirrels in the sanctuary had shown neurological signs, and of these, approximately 50% had survived. The squirrel submitted had a 2-day history of illness that started as limb paresis, lethargy, incoordination, falling and muscle twitching. Within 24 hours, this progressed to seizuring, and the squirrel was euthanized.

Gross necropsy lesions were emaciation and red-pink mottling of the lungs. The brain was grossly unremarkable, but **histopathology showed severe nonsuppurative meningoencephalitis**. Lesions were generalized, in both gray and white matter, and consisted of multifocal gliosis with occasional neutrophils, mild lymphocytic perivascular cuffs with small numbers of neutrophils in both neuropil and meninges, marked hypertrophy of endothelial cells, acute mild neuronal and Purkinje cell necrosis, mild satellitosis, and mild acute perivascular hemorrhage. Lesions in other tissues consisted of mild acute multifocal cardiac myofiber degeneration with small numbers of intralesional macrophages and lymphocytes, mild multifocal interstitial lymphocytic nephritis, and severe intestinal cryptosporidiosis. The lung was moderately atelectatic, and interalveolar septa had a mild diffuse increase in mononuclear cells. There were moderate numbers of alveolar macrophages, sublobular infiltrates of neutrophils in alveoli, type 2 cell hyperplasia, and small numbers of multinucleated and syncytial cells in alveoli. Rare bronchioles had focal epithelial hyperplasia and neutrophils in the lumen.

The fluorescent antibody test for rabies was negative. Immunohistochemistry of the brain showed abundant staining for *West Nile virus* antigen. No specific immunohistochemical staining for *Canine distemper virus* antigen was seen in sections of lung, small intestine, or mesenteric lymph node. While the lung lesion was suggestive of a viral infection, due to financial constraints, there was no additional testing done to confirm the cause of the interstitial alveolitis. Previous reports also describe inconsistently finding myocardial lesions, including multifocal myocardial necrosis, multifocal nonsuppurative myocarditis, fibrosis or myocyte vacuolation, and kidney lesions consisting of multifocal lymphoplasmacytic nephritis. In our case, the mild acute cardiac myonecrosis and the nephritis may have been due to WNV, but immunohistochemistry and PCR testing of the heart and kidneys were not done to confirm this. **Some authors suggest that the optimal tissues to collect from squirrels suspected of WNV infection for histology, IHC or PCR testing are the kidney, brain and heart.**

WNV infection has been reported in North America since 1999. In some regions, tree squirrels have been reported to have a high seroprevalence rate, suggesting that natural exposure is common. From the limited amount of information available, it appears that there likely are differences among species of squirrels in their susceptibility to the virus.

In Ontario, only a small number of cases of WNV have been diagnosed in squirrels previously. These consisted of 6 cases in grey squirrels (*S. carolinensis*) and 2 in red squirrels (*Tamiasciurus hudsonicus*), including animals from both wildlife rehabilitation centers and the wild. The clinical signs and histopathology in our case are similar to those in previous reports. *AHL*

Infectious bronchitis virus testing update

Sarah Hoyland, Marina Brash, Emily Martin, Davor Ojkic

The AHL has evaluated and implemented a PCR test, originally developed at the University of Georgia, for rapid identification of *Infectious bronchitis virus* (IBV). This is a real-time PCR test with an analytical sensitivity of 97.90% and specificity of 100.00%. Our recommendations **for IBV diagnosis** are to submit either pooled tracheal swabs (5 live birds per pool) or tissues from dead birds (separate pools of tracheas, lungs, cecal tonsils – 5 birds per pool).

Submitting multiple pools will result in increased likelihood of detecting the virus. The **IBV real-time PCR** is a rapid detection assay and does not determine the virus strain. Follow-up testing involving **virus isolation** and **virus typing by conventional PCR and sequencing** can be done, but this testing may take several additional weeks because the virus may need to be isolated before typing can be attempted. *AHL*

Goiter in finches

Emily Martin

In the fall of 2011, 2 related cases of zebra finches (*Taeniopygia guttata*) with a history of sudden death were submitted to the AHL for post mortem and histopathology. On gross post mortem examination, there were few significant findings. On histopathology, there were few changes in the majority of tissues except for the thyroid glands that had poor colloid formation (Figure 1) and hyperplasia of the follicular epithelium (Figure 2) consistent with a diagnosis of thyroid hyperplasia or goiter.

In birds, the thyroid glands are paired red-brown oval organs located just cranial to the junction of the common carotid artery and subclavian artery. They consist of multiple epithelial lined follicles that contain homogeneous colloid composed of the iodinated protein thyroglobulin. The major difference between mammalian and avian thyroid glands is that calcitonin cells are parafollicular in mammals, but in birds these cells are in paired ultimobranchial glands that lie caudal to the parathyroid glands.

Avian thyroid function is essentially equivalent to mammals, and they have equivalent thyroid hormones (thyroxine/tetraiodothyronine/T4, triiodothyronine/T3). Iodide is actively transported into the thyroid gland from the blood and iodide content in the thyroid is proportional to dietary iodide intake. Circulating thyroid hormones and thyroid gland hormone content are essentially unaffected by a wide range of iodide intakes. In adult birds with adequate iodide intake, the thyroid gland contains primarily T4 with less or undetectable amounts of T3. When iodide is limited, the T3/T4 ratio is increased and total hormone stores are decreased.

Thyroid hormone affects metabolism, thermoregulation, oxygen consumption, energy supply, liver glycogen storage, plasma glucose, growth, hatching, moult, and reproduction. Environmental factors that affect the thyroid glands include temperature and food availability/composition.

Seeds are a very poor source of minerals and trace elements, and **birds on a seed diet require supplementation or they are prone to dietary deficiencies including iodine deficiency.** Clinical signs of deficiency include dyspnea (audible whistling or squeak due to gland pressure on the trachea), regurgitation of food (due to gland pressure on the esophagus), circulatory problems (due to compression of the heart or great vessels), and lack of activity.

Diets should be evaluated to make sure they are completely formulated and include trace elements such as iodine. Supplements for drinking water are also available. The possibility of the diet containing potential goitrogens (antithyroid substances; e.g., *Brassica* plants, rapeseed, etc.) should also be a consideration and ruled out as part of the history.

When performing necropsies on pet birds, it is important to examine the thyroid glands and consider submitting them for histopathology. If you are submitting a small whole bird fixed in formalin for histopathology, it is important to slit open the celomic cavity to allow perfusion and fixation of the internal organs. We can locate the thyroid glands when the tissues are trimmed at AHL.

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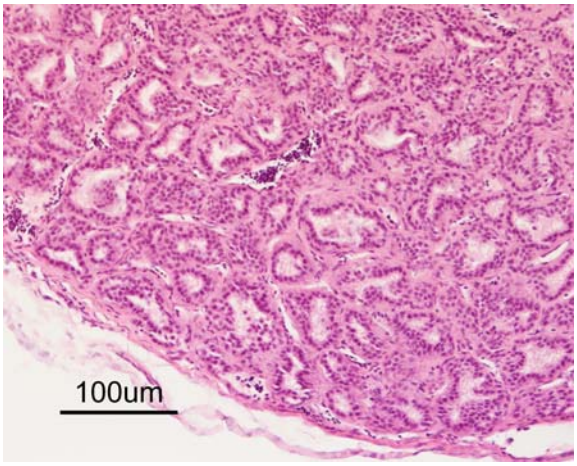

Figure 1. Histology of the thyroid gland demonstrating poor colloid storage within the follicles (20X).

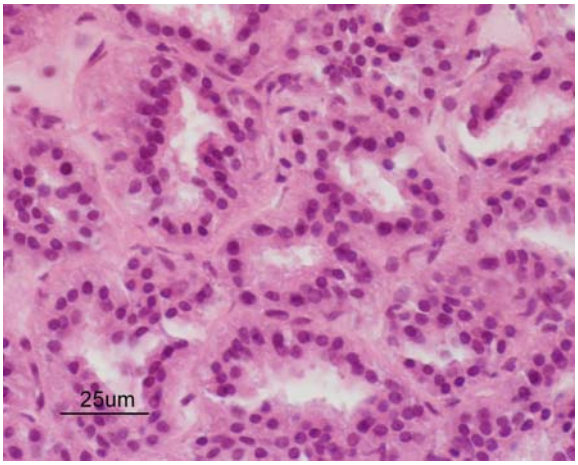

Figure 2. Higher magnification of the thyroid gland demonstrating thyroid follicles with hyperplastic follicular epithelium and poor colloid storage (60X).

HORSES

Equid herpesvirus-1 update

Susy Carman, Beverly McEwen, Josepha DeLay, Murray Hazlett, Margaret Stalker

Equid herpesvirus 1 (EHV-1) is endemic in horse populations, with most horses having antibody to EHV-1 by one year of age. Life-long latent infection is common, with recrudescence and nasal shedding occurring with stress, as a result of transport, showing, or addition of new members to a group. EHV-1 causes respiratory disease, abortions, and is occasionally identified in weak neonatal foals infected *in utero*.

Recrudescence of EHV-1 infection in a pregnant mare may result in abortion. Fetal fluids and placenta are a rich source of infectious virus, and all contaminated fomites should be disinfected. **When pregnant ponies were experimentally infected with EHV-1, EHV-1 could not be identified in 14/32 of aborted fetuses.** One of the 14 mares was euthanized immediately after abortion. Post-mortem examination revealed severe, widespread vasculitis, with thrombosis and ischemic damage in the endometrium, with replication of EHV-1 in endothelial cells. Therefore EHV-1 abortion can occur due to endometrial damage alone, without the establishment of a fetal infection. A negative PCR test result on fetal tissues does not rule out EHV-1 as the cause of abortion.

EHV-1 can also be a cause of serious neurologic disease, with neuropathogenic strains of the virus more likely to induce neurological disease than non-neuropathogenic strains. A real-time PCR is used to identify and differentiate these 2 biotypes. **When archived EHV-1 abortion strains from Central Kentucky were evaluated, 19.4% of the 31 viruses isolated during 2000-2006 were found to be the neuropathogenic strain, which is an increase in prevalence from the 14.4% for 90 EHV-1 viruses recovered in the 1990s.** Testing of 12 archived randomly selected EHV-1 strains, isolated from aborted equine fetuses presented to the AHL between 2003 and 2007, identified only 1 as a neuropathogenic strain, and the remaining 11 strains as non-neuropathogenic.

EHV-1 can be detected by virus isolation and by identifying viral antigens with fluorescent antibody (FA) or immunohistochemistry (IHC) tests. Viral nucleic acid is detected with PCR. The proportion of AHL equine pathology cases found to be EHV-1 positive by FA, IHC, or PCR testing was greater in 2011 (9%) than 2010 (4%), but was less than in 2009 (14%) (Figure 1). The majority of 2011 cases were abortions (n= 8), with a single case of premature delivery of a weak neonate, and 1 case of encephalitis in a mature horse identified in March 2011.

Since 2007, 4 neuropathogenic and 11 non-neuropathogenic strains of EHV-1 have been confirmed at the AHL using PCR, with the **most recent neuropathogenic cases occurring in March 2011 and in January 2012**. *AHL*

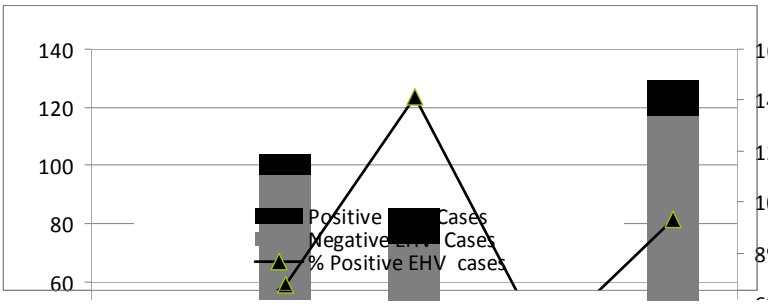

Figure 1. Proportion of AHL equine pathology cases positive for EHV-1 when tested by FA, IHC, or PCR, 2007-2011.

Streptococcus equi subsp. *equi* vaccine and wild type strain can now be differentiated at the AHL *Hugh Cai, Ana Rita Rebelo, Durda Slavic*

The AHL now offers molecular typing assay to differentiate vaccine and wild-type strains of *Streptococcus equi* subsp. *equi*. The assays were adopted from the Veterinary Diagnostic Laboratory, Illinois.

The currently available modified-live vaccine (MLV) is an acapsular mutant that has a predominantly dry colony morphology but can produce encapsulated isolates from reconstituted vaccine as well as serial passages of dry morphology colonies on enriched media. Therefore it is not reliable to differentiate wild type and MLV strains based on colony morphology. Recently, molecular biology methods have been described for the differentiation of vaccine strains and wild-type strains by sequence analysis of seM and szp genes.

Continued on p. 11 ⟶

Ontario Racing Commission Death Registry: 2003-2011 necropsy summaries *Josepha DeLay*

The Ontario Racing Commission Death Registry has been in place since 2003 and continues to provide excellent data regarding the causes of morbidity and mortality in racehorses in this province. Necropsy requests by the ORC have become more selective in the past 4 years, with a shift in emphasis to non-fracture cases and with increased complexity. Summaries of necropsy submissions to the Animal Health Laboratory under this program and diagnoses by body system for these cases are provided in the following tables. *AHL*

Table 1. Breed distribution of ORC Death Registry submissions to the AHL, 2003-2011

Breed / Year	Standard-bred	Thoroughbred	Quarter Horse	Total
2003	67 (54%)	58 (46%)	0	**125**
2004	82 (58%)	60 (42%)	0	**142**
2005	59 (54%)	51 (46%)	0	**110**
2006	58 (54%)	47 (44%)	2 (2%)	**107**
2007	66 (54%)	53(43%)	3(3%)	**122**
2008	27 (53%)	24(47%)	0	**51**
2009	28 (62%)	16 (36%)	1 (2%)	**45**
2010	22 (69%)	8 (25%)	2 (6%)	**32**
2011	24 (52%)	18 (39%)	4 (9%)	**46**

Table 2. Necropsy diagnoses of ORC Death Registry submissions by body system, 2003-2011. Note change in emphasis of case selection for necropsy submissions beginning in 2008, as mentioned in text above.
(*CNS cases in 2011 included 2 horses with WNV encephalomyelitis, 2 horses with equine protozoal myelitis (EPM), and 1 horse with EHV-1 encephalomyelitis).

Diagnosis by body system:	2003	2004	2005	2006	2007	2008	2009	2010	2011
Fracture / limbs	53 (42%)	69 (49%)	48 (44%)	42 (39%)	54 (44%)	16 (31%)	4 (9%)	9 (28%)	6 (13%)
Fracture / other	10	4	7	13	10	5	0	3	6
Non-fracture musculoskeletal	8	6	6	8	6	5	2	3	1
Gastrointestinal	15	19	17	16	18	5	4	7	5
Respiratory (including EIPH)	21	17	9	11	16	9	21	6	9
Cardiovascular	5	6	5	5	2	4	6	2	4
CNS	6	11	7	4	1	1	2	0	5*
Integumentary	0	0	1	2	2	1	1	0	0
Renal	0	2	0	0	2	0	1	0	0
Hematopoeitic	2	1	1	0	0	0	0	0	0
Whole body conditions	1	7	5	2	9	0	4	0	6
Cause of death undetermined	4 (3.2%)	0 (0%)	4 (3.6%)	4 (3.7%)	2 (1.6%)	5 (9.8%)	0 (0%)	2 (6%)	4 (9%)
Total	**125**	**142**	**110**	**107**	**122**	**51**	**45**	**32**	**46**

Streptococcus equi sequencing - continued

Pure cultures of *S. equi* ssp. *equi* can be submitted to the AHL Molecular Biology Lab for *S. equi* sequencing typing (test code "sequity"). Alternatively clinical samples from sick horses can be submitted to the AHL Bacteriology Lab for bacterial culture, and, if isolated, *S. equi* subsp. *equi* culture can be forwarded to the Molecular Biology Lab to determine if it is a wild type or MLV strain. *AHL*

COMPANION ANIMALS

Small animal diagnostic services at AHL-Kemptville

Andrew Brooks and Jan Shapiro

The full range of small animal diagnostic services at the AHL is provided through our Kemptville laboratory. AHL-Kemptville is located on the Kemptville campus of the University of Guelph, approximately 40 km south of Ottawa. Although livestock, poultry, and horses make up the majority of submissions to our Kemptville lab, **a significant proportion of our caseload involves small animals, including pet birds, exotic species and pocket pets**. In general, our cases are submitted from 13 counties that stretch from the Quebec border to the east and north, south and west along the St. Lawrence River to Belleville, as far north as Pembroke, and the area west of the Ottawa River to Algonquin Park.

In addition to necropsy, biopsy and clinical pathology services, **the AHL provides a broad range of expertise in medico-legal pathology, toxicology, microbiology, molecular diagnostics and immunohistochemistry**.

The AHL-Kemptville team:

Left to right: Dr. Jan Shapiro, Dr. Andrew Brooks Amber Couturier, Tom McLean.

Please contact Andrew or Jan for enquiries about diagnostic services and specimen submission. *AHL*

Indications for bone marrow examination *Kristiina Ruotsalo*

Indications for bone marrow evaluation are numerous and include the investigation of atypical cells in peripheral circulation, the diagnostic work up of unexplained persistent cytopenias, the investigation of suspected lymphoproliferative or myeloproliferative disease, and the staging of hematopoietic tumors such as lymphoma.

An EDTA sample for a complete blood count should always be submitted along with the bone marrow sample as this is vital for accurate interpretation.

Bone marrow aspiration is ideal for those diseases in which the whole marrow in affected. Occasionally, focal disease or increased marrow stromal components make bone marrow biopsy preferable. As the need for bone marrow biopsy often cannot be predicted at the time of marrow aspiration, some clinicians routinely collect both samples.

Below is an example of a peripheral blood smear (Figure 1) and bone marrow aspirate (Figure 2) from a 10-year-old, intact male Golden Retriever with a history of lethargy, anorexia, and forelimb swelling. CBC revealed the following: hemoglobin of 98 g/L with no evidence of polychromatic erythrocytes, a WBC of 1.0 $X10^9$/L and a platelet count of 36 $X10^9$/L. The rare neutrophils and lymphocytes identified within the blood smear were morphologically unremarkable. Given the unexplained pancytopenia, bone marrow aspiration was undertaken. The aspirate was highly cellular with almost complete replacement of normal hematopoietic tissue by a population of large immature round cells most consistent with a lymphoid origin. **A diagnosis of acute leukemia, most likely of lymphoid origin was made**. *AHL*

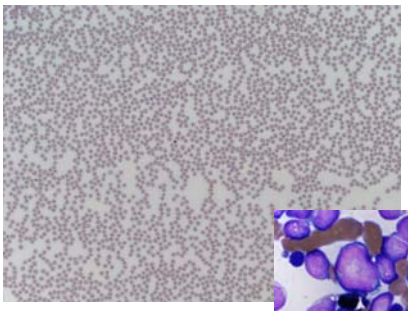

Fig. 1. Peripheral blood smear, pancytopenia.

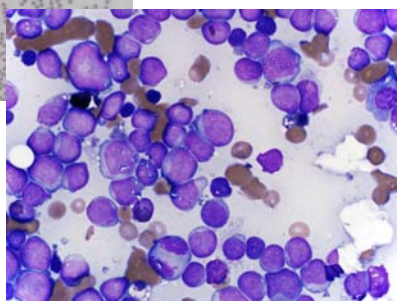

Fig. 2. Bone marrow aspirate, lymphoid proliferation, acute lymphoid leukemia.

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