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Animal Health Laboratory

AHL Newsletter

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In this issue:

Validated *Pasteurella multocida* toxin PCR - swine

Hugh Cai, Davor Ojkic, Jim Fairles

We have now finished validation of a PMT PCR for detection of the toxin gene, as an alternative to ELISA testing. Submission requirements will remain the same as for the ELISA . PMT swabs will be available from AHL as before. Similar to the ELISA, nasal swabs will be pooled in groups of 2, and incubated on a PMT plate for 24 hours. The resulting growth of *Pasteurella* will be evaluated by PCR for the toxin gene.

The cost will be the same as ELISA, $11.00 per swab ($22.00 for a pool of 2) with a turnaround time of 3-8 days. The lab should receive the samples Monday - Wednesday for prompt set-up. *AHL*

Fecal parasite egg counts - equine, ruminant

Mary Lake, Hugh Cai

Fecal egg count testing is typically done to check for anthelmintic resistance/ activity. Pre-treatment fecal samples are taken at the time of, or just before, anthelmintic treatment. Post-treatment samples are taken 7-10 days after anthelmintic treatment. **A significant reduction in egg count should be seen between pre and post samples.**

Fecal egg counts are routinely requested on equine submissions and occasionally requested on ruminant samples. We have simplified the name/code of this test to "**Fecal egg count - code fec**", with results reported as number of eggs per gram of feces (epg). The charge for this test is $18.00. For herd samples, we will charge $18.00 for each of the first two samples, and $15.00 for each additional sample. Turnaround time is 3-5 days.

In the past, we performed a Cornell-Wisconsin centrifugal float (*cflo*) (for low egg counts 0-400) or a Cornell-McMaster dilution float (*comc*) (for egg counts >400) when there was a request for egg count - one or both techniques will now be done as appropriate. *AHL*

Mycoplasma iowae real-time PCR developed and validated - avian

Hugh Cai, Patricia Bell-Rogers, Lois Parker, Arpad Ferencz, Peter Pozder

The AHL is now offering a real-time PCR test for *Mycoplasma iowae* in cloacal swab samples at a fee of $28 per test. The PCR assay is set up weekly unless special arrangements are made.

Infection with *M. iowae* is seen in turkeys, and can occur in other poultry and wild bird species as well. Unlike other avian *Mycoplasma* spp., there is no commercial serological test for *M. iowae*. Culture isolation and identification of the pathogen takes 10-14 days for positive samples, and 21 days to confirm a negative result. Recently, the AHL has developed and validated a *M. iowae* real-time PCR assay. The assay is specific for *M. iowae,* with a detection limit of 30 cfu per cloacal swab sample. In field validation testing of 350 cloacal swab samples, the sensitivity of the PCR was 100%, and the specificity was 99.3% (Table 1). The PCR and culture method were in almost perfect agreement (kappa = 0.97). In addition, unlike culture isolation, the PCR assay seems not to be affected by overgrowth of bacteria. *AHL*

Table 1. Comparison of M. iowae real-time PCR and culture isolation

	Culture positive	Culture negative
PCR positive	38	2*
PCR negative	0	310

*The 2 samples positive by PCR and negative by culture were overgrown with bacteria.

AHL accomplishments in 2006

Awards/Honors:

Keith Harron - Poultry Health Worker of the Year award - presented by the Poultry Industry Council, at the Poultry Health Conference, Kitchener, ON, Nov 14, 2006.

David Hilchie - Lee G. Luna First Time Symposium Presentation Award, National Society for Histotechnology, Phoenix, AZ, Sept 12, 2006.

Grant Maxie - President-elect, 2007, American Association of Veterinary Laboratory Diagnosticians (AAVLD).

New accreditation:

ISO 17025 - April 2006, AHL was accredited by SCC for MOL-180, Real-time scrapie resistance PrP genotyping.

Proficiency panels passed

AAVLD check tests - Bacteriology

CFIA Equine Infectious Anemia proficiency panel/certified analysts - Jane Coventry, Hongwen Fu, Lynn Henry, Sophia Lim, Linda Little, Maria Osuch, Gaye Smith.

CFIA Avian Influenza virus AGID panel - Gaye Smith.

CFIA BSE panel - Jane Coventry, Hongwen Fu.

CFIA CWD panel - Jane Coventry, Hongwen Fu.

USDA *Mycobacterium paratuberculosis* ELISA panel - Hongwen Fu, Sophia Lim.

VLA Quality Assurance (quarterly) - Clinical Pathology, Bacteriology, Toxicology.

New tests added in 2006:

- PMT PCR test.
- Tetracore real-time multiplex PRRSV PCR test.
- Canine TLI (trypsin-like immunoreactivity).
- Diasorin 2-step free T4.
- Endogenous ACTH assay.

Presentations:

American Association of Veterinary Laboratory Diagnosticians (AAVLD), Minneapolis, MN, Oct 12 - 16, 2006:

- Beverly McEwen. Test Validation at the AHL. Invited talk, QA Committee.
- Brent Hoff. Case report - Anatoxin-A in benthic cyanobacteria and associated poisoning in four dogs.
- Durda Slavic. Bacteriology case report, Bacteriology Committee.
- Nick Schrier. Method validation within AHL-Toxicology. Veterinary Analytical Toxicology and Mycotoxins group (meeting co-chaired by Brent Hoff).

Carman S, Hazlett M, DeLay J, Carr N. BVDV Type 1b in alpaca: abortion and persistent infection. BVD 2006 International Conference. BVD Control: The Future is Now, Denver Colorado, Jan 29-31, 2006.

Hilchie D. From critter to cassette and beyond. National Society for Histotechnology, Phoenix, Arizona, Sept 12, 2006.

Hoff B. Invited speaker. Can Vet Med Assoc annual conference, St John's NL, July 7/8, 2006.

- Nutritional and metabolic profile testing of dairy cows.
- Laboratory diagnosis of ketosis and hepatic lipidosis in cattle.
- Case studies in the use of haptoglobin by animal health laboratories.
- Clinical pathology case discussions (with Shelly Burton).

Hoff B. Poisonous plants in Ontario and their effects on animals. Grey-Bruce Veterinary Association, Owen Sound, May 10, 2006.

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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. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the editor.*

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AHL accomplishments in 2006 – continued from page 2.

Posters:

Cai H, Bell-Rogers P, Parker P, Prescott J. Development of a real-time PCR for detection of *Mycoplasma bovis* in bovine milk and lung samples. 4th Intern Vet Vaccines Diagnostics Conf (IVVDC). June 25-29, 2006, Oslo, Norway.

Chen S, Li J, Odumeru J, Lu L, Muckle A, Cai H. Improvement and validation of an antibody microarray method for *Salmonella* serotyping. Intern Assoc Food Protect (IAFP), Calgary, Alberta, August 13 - 16, 2006.

Ilha MRS, Burgess H, Rodriguez A, Slavic D, McEwen B, Baird J. *Salmonella typhimurium* var. Copenhagen septicemia as a cause of meningoencephalomyelitis/encephalitis in neonatal foals. AAVLD, Minneapolis, MN, Oct 12 - 16, 2006.

Olsen CW, Waller J, Karasin AI, Carman S. Genetic analyses of H3N2 swine influenza viruses isolated in Canada in 2005-2006. CRWAD 2006 Chicago Il.

Ridpath JF, Neill JD, Vilcek S, Dubovi EJ, Carman S. Epidemic of acute BVDV in Quebec, Ontario and New York State linked to the same strain of BVDV2. BVDV Symposium: BVD control: the future is now. Denver, Colorado, 2006.

Waller J, Karasin AI, Carman S, Olsen CW. Phylogenetic analyses of H3N2 and H1N1 swine influenza viruses isolated in Ontario in 2005-2006. CRWAD 2006 Chicago Il.

Peer-reviewed publications:

Bolam CJ, Hurtig MB, Cruz A, McEwen BJ. Characterization of experimentally induced post-traumatic osteoarthritis in the medial femorotibial joint of horses. Am J Vet Res 2006;67:433-447.

Carman S, McEwen B, DeLay J, van Dreumel T, Lusis P, Cai H, Fairles J. Porcine circovirus-2 associated disease in swine in Ontario (2004 to 2005). Can Vet J 2006;47:761-762.

Dewey C, Carman S. Practice tip - bleeding boars. Swine Health Production 2006;14:267-268.

Gagea MI, Bateman KG, van Dreumel T, McEwen BJ, Carman S, Archambault M, Shanahan RA, Caswell JL. Diseases and pathogens causing mortality in Ontario beef feedlots. J Vet Diagn Invest 2006;18:18-28.

Gagea MI, Bateman KG, Shanahan RA, van Dreumel T, McEwen BJ, Carman S, Archambault M, Caswell JL. Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. J Vet Diagn Invest 2006;18:29-40.

Gomis S, Goodhope AR, Ojkic AD, Wilson P. Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. Avian Dis 2006;50:550-555.

Grgic H, Philippe C, Ojkic D, Nagy E. Vertical transmission of fowl adenoviruses. Can J Vet Res 2006;70:230-233.

Hoff, B, Thomson G, Graham K. Neurotoxic cyanobacterium (blue-green alga) toxicosis in Ontario. Can Vet J 2007;48:147.

Karasin AI, Carman S, Olsen CW. Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada, 2003-2005. J Clin Microbiol 2006;44:1123-1126.

Meyer J, DeLay J, Bienzle D. Clinical, laboratory, and histopathologic features of equine lymphoma. Vet Pathol 2006;43:914-924.

Ojkic D, Swinton J, Vallieres M, Martin E, Shapiro J, Sanei B, Binnington B. Characterization of field isolates of infectious laryngotracheitis virus from Ontario. Avian Pathol 2006;35:286-292.

Olsen CW, Karasin AI, Carman S, Li Y, Bastien N, Ojkic D, Alves D, Charbonneau G, Henning B, Low D, Burton L, Broukhanski G. Isolation of triple reassortant H3N2 influenza A viruses from pigs, turkeys, and a swine farm worker in Canada. Emerg Infect Dis 2006;12:1132-1135.

Peregrine AS, Martin SW, Hopwood DA, Duffield TF, McEwen B, Hobson JC, Hietala SK. *Neospora caninum* and *Leptospira* serovar serostatus in dairy cattle in Ontario. Can Vet J 2006;47:467-470.

Peregrine AS, McEwen B, Bienzle D, Koch TG, Weese JS. Larval cyathostominosis in horses in Ontario: an emerging disease? Can Vet J 2006;47:80-82.

Philippe C, Grgic H, Ojkic D, Nagy E. Serologic monitoring of a broiler breeder flock previously affected by inclusion body hepatitis and testing the progeny for vertical virus transmission. Can J Vet Res 2006; in press.

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TSE Testing at the Animal Health Laboratory

Jim Fairles, Davor Ojkic

The AHL is a partner in the Canadian TSE Laboratory Network and is approved by CFIA for TSE testing as noted in the LabNote on our website: http://www.labservices.uoguelph.ca/units/ahl/documents/LabNote07-TSE.pdf

We are involved in various programs including:

- CFIA BSE surveillance
- OMAFRA Chronic Wasting Disease Surveillance Project in Farmed Deer and Elk (now extended to March 2008)
  - http://www.omafra.gov.on.ca/english/livestock/alternat/facts/infoCWDsurveillanceproject1.htm
  - http://www.omafra.gov.on.ca/english/livestock/alternat/facts/info_chronic_wasting_update.htm
- Ontario Ministry of Natural Resources CWD surveillance
  - http://www.mnr.gov.on.ca/MNR/hunting/cwd/plan.html
- Scrapie Canada - Scrapie Genotyping and Flock Certification Program
  - http://www.scrapiecanada.ca/home.html

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Selected zoonotic pathogens and diseases identified at the AHL, 2002 - 2006

Beverly McEwen, Durda Slavic, Davor Ojkic, Susy Carman, Josepha DeLay, Tony van Dreumel

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 (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. *West Nile virus* continues to be identified by PCR in non-domestic species and occasionally in horses by IgM ELISA, histology and immunohistochemistry. Multisystemic disease due to *Halicephalobus gingivalis,* a nematode, was identified in one horse submitted for necropsy. *AHL*

Table 1. Selected zoonotic pathogens isolated and/or identified at the AHL, 2002-2006

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	**2006**	2005	2004	2003	2002
Bartonella henselae											**0**	1	0		
Bordetella bronchiseptica		48	4					4		12	**68**	70	78	84	
Borrelia burgdorferi											**0**	1			
Campylobacter coli/ jejuni/ fetus subsp. fetus				14				13		6	**33**	14	26	31	33
Chlamydophila sp.	1			17	2					2	**21**	6	6	19	10
Coxiella burnetii (Q fever)				2	6						**8**	6	4	6	6
Cryptosporidium sp.	169	4	1	3	2			2	1	3	**185**	201	61	129	199
Eastern equine encephalitis virus											**0**	0	2	10	0
Francisella tularensis											**0**	0	1	1	1
Giardia sp.	9							27			**36**	19	13	10	4
Listeria monocytogenes	5			4	4					2	**15**	16	34	27	37
Methicillin-resistant *Staphylococcus aureus*			1					3	1	3	**8**	24	133	73	13
Mycobacterium bovis											**0**	0	0	0	1
Rabies											**0**	1	0	0	0
Salmonella sp.	167	136	193	4		187	62	3		95	**847**	836	640	824	716
Streptococcus suis	8	397		2		2					**409**	608	447	392	477
Streptococcus equisimilis		72	11	3				6		4	**96**	82	140	111	144
Streptococcus zooepidemicus	7	2	159								**168**	188	186	211	222
Toxoplasma sp.				7	2				2	2	**13**	6	10	5	12
West Nile virus			1							148	**149**	100	158	173	91
Yersinia enterocolitica		1						3			**4**	3	8	8	5

Table 2. *Leptospira spp*. seropositive samples (≥1:320) identified at AHL, 2002 – 2006, microscopic agglutination test (MAT)

***Leptospira spp*. serovar**	Bovine	Swine	Equine	Canine	Feline	Other & not specified	**2006**	2005	2004	2003	2002
L. autumnalis	3			188		48	**191**	215	226	122	46
L. bratislava		67	55	170		2	**292**	361	329	151	139
L .canicola	1			35			**36**	43	56	17	4
L. grippotyphosa	1	24	10	173		2	**208**	160	196	76	29
L. hardjo	23			7			**30**	51	60	34	30
L. icterohaemorrhagiae	17			61			**78**	58	111	122	163
L. pomona	16	8	18	93		1	**135**	148	128	122	105
Total	**61**	**99**	**83**	**727**	**0**	**53**	**970**	1036	1106	644	516

AHL Lab Reports

RUMINANTS

Highlights of small ruminant diagnoses, 2005 and 2006

Janet L. Shapiro, Brian Binnington, Beverly McEwen

Pathology submissions of small ruminants to the Animal Health Laboratory, Guelph and Kemptville from January 1, 2005 - December 31, 2006 were reviewed to identify the most prevalent diagnoses associated with mortality in different age groups.

Aborted and stillborn lambs and kids

Infectious and idiopathic inflammatory disease was the predominant cause of aborted and stillborn offspring. Of the infectious causes identified, the zoonotic agents *Chlamydophila abortus, Toxoplasma gondii, Campylobacter spp,* and *Coxiella burnetii* predominated, and were identified in over 40% of abortion and stillbirth submissions. Several diagnoses of congenital goiter were made in both sheep and goat submissions; affected flocks and herds reported multiple cases of abortion, stillborn or weak neonates.

Postnatal sheep and goats

Gastrointestinal tract (GIT) disease was the most common diagnosis.

Parasitic diseases, including coccidiosis, hemonchosis and mixed gastrointestinal parasite infections comprised almost 50% of the GIT diagnoses. **Hemonchosis** was diagnosed frequently in the summer months of 2006, for which the hot wet weather was considered a contributing factor; however dead animals were often from flocks and herds which had been repeatedly and recently dewormed. Drs. Paula Menzies and Andrew Peregrine have suggested that this may have been due to incorrect drug dosage or ineffective technique for administration of anthelmintic, or use of ineffective or unlicensed products. **Cryptosporidiosis** was a cause of fatal diarrhea in lambs and kids 5-10 d of age, and in 1-2 mo old kids from a herd in which caprine arthritis encephalitis (CAE) was endemic. **Johne's disease** was diagnosed in both sheep and goat submissions. Acute or peracute disease caused by **enterotoxemia** due to *Clostridium perfringens* type D was diagnosed with equal frequency in sheep and goats in good body condition from 3 d to 6 mo of age, and in a mature doe that died 1 wk after a sudden change in feed. In submissions for which a vaccination history was provided, affected animals were not vaccinated against clostridial diseases. **Rumenitis** due to grain overload was infrequently diagnosed in both sheep flocks and goat herds.

Respiratory tract disease was the second most common diagnosis in all ages of sheep and goats. Fatal acute pneumonia, or less frequently septicemia, caused by ***Mannheimia haemolytica*** was a common diagnosis in lambs and kids under 6 wk of age. This agent was also cultured from a small number of older animals, often in combination with other bacterial pathogens. In lambs, a few cases of interstitial pneumonia histologically consistent with infection with respiratory syncytial virus (RSV) or parainfluenza 3 virus (PI3) were diagnosed. Sporadic cases of **nasal adenocarcinoma** were diagnosed in flocks in both eastern and western Ontario.

Chronic copper toxicity was diagnosed in flocks with multiple morbidity and mortality associated with toxic hepatopathy of ewes, as well as other infectious disease problems in younger stock. In 1 flock, the source of copper was a sheep feed formulation error. In another flock, the source was believed to be a grain ration formulated for dairy calves.

Listeriosis, polioencephalomalacia, brain abscesses and caprine arthritis-encephalitis (CAE) were the most common diagnoses of the CNS. **Listeriosis** was a frequent diagnosis in both sheep and goats with neurological signs; affected herds and flocks reported multiple mortalities. In the goat herds, there was often a history of feeding ensiled or spoiled feed. Inflammatory lesions in the lung and/or the CNS consistent with a diagnosis of **CAE** was made in 4 goat herds that reported multiple affected animals. In kids 3-wk to 4-mo old, the clinical history was chronic pneumonia or neurological signs. Mature does had a history of stiffness, decreased milk production and wasting.

Nutritional myopathy was the most frequently diagnosed disease of skeletal and cardiac muscle. Affected sheep and goats ranged from 5 d of age to adult, and the history provided with the submissions did not indicate exposure to ionophores.

Other diagnoses that were relatively common were **caseous lymphadenitis** affecting multiple external or internal lymph nodes and/or lungs in sheep, and **bacterial mastitis**. *AHL*

Acute toxicity of Star-of-Bethlehem (*Ornithogalum umbellatum)* in a herd of beef cattle

Brent Hoff, Gary Thomson, Randy Dingwell

In late fall 2006, 10 of 20 head of beef cattle died as a result of eating discarded Star-of-Bethlehem bulbs (*Ornithogalum umbellatum),* a plant containing cardenolides (cardiac glycosides) similar to oleander, lily-of-the-valley, milkweed and foxglove. Three animals were found dead the first day. Several other animals were restless with incoordinated movements, dyspnea and bloody diarrhea. Two more animals died the next morning, and 5 more over the next few days.

The day before the deaths began; the animals were let out in the barnyard, in order to clean out the pen in the barn. The animals had access to 2 wheelbarrow loads of bulbs discarded from a cut-flower market garden on the premises. The bulbs were discarded on the manure pile, after a wet fall where the bulbs were graded and cleaned of wet soil and soft bulbs discarded. During a typical fall, the discarded bulbs would have been left in the field and plowed in later in the fall.

Clinical pathology data on one of the animals revealed neutropenia and hemoconcentration. The animal also had mild hypocalcemia and a mild increase in hepatic enzymes.

At necropsy, all 3 animals had acute, widespread, intestinal congestion and hemorrhage, with mucosal erosion. Endocardial and epicardial hemorrhage was also noted. Histopathology of sections of the myocardium showed numerous contraction bands in all animals.

Cardiac glycosides are found in all parts of the plant, especially the bulbs. Generally, only very small quantities of the plant must be ingested to produce poisoning. In cattle and horses, as little as 0.005% of body weight is reported as lethal.

Animals consuming the plants develop **primarily cardiac and digestive disturbances before death**. Glycosides act directly on the gastrointestinal tract causing intestinal hemorrhage, abdominal pain and diarrhea. Cattle especially, and less often horses and dogs, consuming cardiac glycoside-containing plants are often found dead because of the profound cardiac effects of the toxins. Cardenolides inhibit the cellular membrane sodium-potassium pump, resulting in a progressive decrease in electrical conductivity through the heart causing a variety of cardiac dysrhythmias and heart block, including ventricular tachycardia and first- and second-degree heart block.

Although typically unpalatable and not likely consumed in a good pasture, poisoning of livestock is quite possible through contamination of fields harvested for animal feeds and, as in this case, through accidental ingestion of bulbs from a cut-flower market garden. *AHL*

Figure 1. Star-of-Bethlehem bulb.

Figure 2. Star-of-Bethlehem flower.

AVIAN SPECIES

Summary of pigeon necropsy diagnoses, 2005- 2006

Marina Brash, Brian Binnington, Emily Martin, Beverly McEwen, Mirjana Savic, Jan Shapiro, Katie Welch

Cases of pigeons submitted to the Animal Health Laboratory have almost doubled from 2005 (n = 22) to 2006 (n = 41). The majority of submissions were pigeons under 6 months of age. At necropsy, few specific lesions were identified and ancillary testing including bacteriology, virology, serology and histology were required for diagnoses. Most of the submissions yielded multiple diagnoses.

The majority of cases in 2006 (32 of 41) were either positive for or suspicious of *Pigeon circovirus* (PiCV) infection with concurrent marked bursal lymphoid depletion. **This diagnosis requires histological examination of the bursa and identification of the typical circoviral inclusions.** In cases suspicious of PiCV, the bursae were depleted of lymphoid cells, but we were unable to confirm the presence of the viral inclusions. This may be due to the late stage of infection and lack of suitable cells for continued viral replication. *Pigeon circovirus* is thought to be immunosuppressive, resulting in birds developing secondary infections including those with viral (herpesvirus, adenovirus), fungal and bacterial causes. The birds tend to succumb to these infections. The diagnosis of PiCV infection can only be made in immature birds as the bursa naturally involutes with sexual maturity.

There was a dramatic increase in the diagnosis of pigeon paramyxovirus-1 (PMV-1) infection in 2006 (n = 19). Pigeon paramyxovirus is closely related to Newcastle disease virus (NDV), and the highly pathogenic form of NDV causes a federally reportable disease. The Ontario Ministry of Agriculture, Food and Rural Affairs recently forwarded a disease alert to chicken and turkey producers about the increase in the frequency of pigeon PMV-1 in pigeon flocks as this poses a potential risk for commercial poultry flocks. Biosecurity is important in preventing exposure to unwanted viruses. This includes employees and equipment from commercial flocks not having either direct or indirect contact with both wild and commercial pigeons. **A vaccine is available to help manage this disease.**

Other viral infections identified (n = 11) were those with inclusion bodies suggestive of either adenoviruses or herpesviruses involving various body systems including bursa, liver, kidney, oral cavity, pharynx, esophagus and crop. **Viral infections of the upper digestive system must be included in the differential diagnoses for oral lesions along with trichomoniasis (canker) and crop mycosis (thrush).** If birds are being treated for canker and do not respond to treatment, a viral etiology must be considered. There are no vaccines available to prevent these other viral infections. New introductions or mixing of birds from multiple sources provides an excellent opportunity to introduce these viruses into lofts, therefore biosecurity measures including quarantine of new introductions are important management strategies.

Intestinal parasites identified (n = 10) included *Capillaria* spp (threadworms) and *Ascaridia* spp (roundworms). In some birds, the roundworm burden was so high that the intestines were bulging with roundworms. Adult roundworms are visible to the naked eye. Threadworms are more difficult to identify at necropsy but eggs are identified on fecal flotation and worms are seen in intestinal sections on histology. A few cases of intestinal coccidiosis / coccidiasis were seen.

Mycotic pneumonia and airsacculitis with *Aspergillus* spp, and bacterial pneumonia and airsacculitis with *E. coli,* were seen in 7 submissions. Heightened susceptibility to mycotic and bacterial agents is likely related to the negative impact of the PiCV infection on the immune system. Crop mycosis with *Candida albicans* (thrush*)* and trichomoniasis (canker) were also diagnosed in some birds. Given the immunosuppressive effect of the PiCV infection, these infections are to be expected. The use of broad -spectrum antimicrobials can also result in increased incidence of crop mycosis.

Only a few cases of salmonellosis due to *Salmonella enterica* Typhimurium have been diagnosed in pigeons in the last couple of years. There has been a reduction in the isolation of *S.* Typhimurium from pigeons submitted to the lab over these last few years. This is good news for the industry as a whole.

When disease problems arise, early intervention may help minimize morbidity and/or mortality. **We request submission of 3 non-medicated birds, live or fresh dead, that are demonstrating the clinical signs of the problem to be investigated.** Information including flock size, number sick, number dead, clinical signs, duration of problem, vaccinations, other treatments, and tentative diagnoses helps us select the most appropriate tests. Typically, these pigeon submissions have several diagnoses but some are more significant to the flock as a whole and others are applicable to the individual bird.

The AHL provides diagnostic services to help field veterinarians identify and manage individual flock problems. If you have any questions about submissions, please contact us at the AHL. *AHL*

SWINE

Porcine circovirus type 2 associated disease and co-infections

Beverly McEwen, Susy Carman, Jim Fairles, Durda Slavic, Hugh Cai

Pathology cases of *Porcine circovirus* type 2 associated disease (PCVAD) increased to 408 cases in 2006, compared to a total of 574 cases from 1998-2005. A summary of data from the last 8 years identified other co-pathogens occurring in cases of PCVAD: *Porcine reproductive and respiratory syndrome virus* (PRRSV) (38%), *Streptococcus suis* (16%), *Pasteurella multocida* (11%), *Mycoplasma hyopneumoniae* (9%), *Haemophilus parasuis* (5%), and swine influenza virus (SIV) (5%).

In 2006, **PCV-2 combined with PRRSV alone, or in combination with SIV, *M. hyopneumoniae* or bacteria collectively accounted for 64% of the cases in which co-infections were identified** (Table 1). Other co-infections with SIV, *M. hyopneumoniae,* and/or bacteria comprised the remaining 36% of the cases.

The relative frequency of cases where PCV-2 was the only pathogen identified has not changed significantly since 1998. The cases in which PCV-2 was the only agent identified were not included due to the frequent practitioner bias for selection of circovirus-specific tests only. The true proportion of PCVAD cases where PCV-2 is truly the sole pathogen is unknown. *AHL*

Table 1. PCVAD and co-infections identified in 2006, AHL pathology cases.

Pathogens identified	**% of cases**
PCV-2 + PRRSV	38%
PCV-2 + PRRSV + SIV	4%
PCV-2 + PRRSV + *M. hyopneumoniae*	3%
PCV-2 + PRRSV + bacteria	19%
PCV-2 + SIV	2%
PCV-2 + SIV + *M. hyopneumoniae*	1%
PCV-2 + SIV + bacteria	3%
PCV-2 + *M. hyopneumoniae*	5%
PCV-2 + bacteria	25%

Cellulitis and necrotizing myositis associated with *Clostridium septicum* infection in grow/finish pigs.

Margaret J. Stalker, Christa A. Arsenault, Durda Slavic

Three, 50-65 kg pigs were submitted for postmortem examination to investigate the cause of ongoing low-to-moderate level losses occurring on a 850 sow farrow-to-finish farm over the past several years. The submitted pigs were typical of the problem, and exhibited remarkably swollen, irregular discoloured areas over the right shoulder and neck regions, surrounded by a zone of hemorrhage (Fig. 1). The pigs also had numerous scabbed skin wounds and excoriations on the thorax, neck and ears.

Postmortem revealed marked subcutaneous edema of the shoulder and neck regions. Adjacent muscle was hemorrhagic, with dry, friable and crepitant areas; some sections of muscle floated in formalin. Fluorescent antibody testing of direct impression smears made from multiple sections of subcutaneous tissue and muscle were positive for *Clostridium septicum* in moderate numbers from all 3 pigs; low numbers of *C. sordellii* were also present. Histology of the lesions revealed marked subcutaneous edema with necrosis of muscle fibers in adjacent skeletal muscle bundles, a minimal inflammatory response, and low numbers of large bacilli present around the periphery of the lesions. One pig also had focally extensive fibrinohemorrhagic pleuritis, and all 3 had fibrin thrombi in pulmonary alveolar capillaries; *Actinobacillus suis* was isolated in moderate-to-large numbers from the lungs of all 3, likely contributing to the cause of death.

Cellulitis and necrotizing myositis are typical lesions caused by wound infection by various species of *Clostridium*. **In swine, *C. septicum* is the primary pathogen causing gas gangrene and malignant edema, although other clostridial species, including *C. sordellii* as in this case, may also play a role.** Swine, horses and ruminants are highly susceptible to these infections, whereas carnivores are rarely affected. These bacteria are common in the environment where they are found as resistant spores in soil.

Contaminated deep wounds and areas of devitalized muscle provide an ideal environment for clostridial proliferation, and elaboration of potent hemolytic and necrotizing toxins that cause additional local tissue damage, and may result in systemic toxemia and rapid death within 24-48 h of infection. Predisposing deep wounds include castration sites, various penetrating wounds, or particularly in swine, inoculation sites. The underlying injury in this case was initially suspected to be vaccination sites (right shoulder), but cases have also occurred in unvaccinated individuals. Mixing and overstocking with associated fighting may also have provided sufficient trauma in these cases, as evidenced by extensive skin wounding over the affected areas. *AHL*

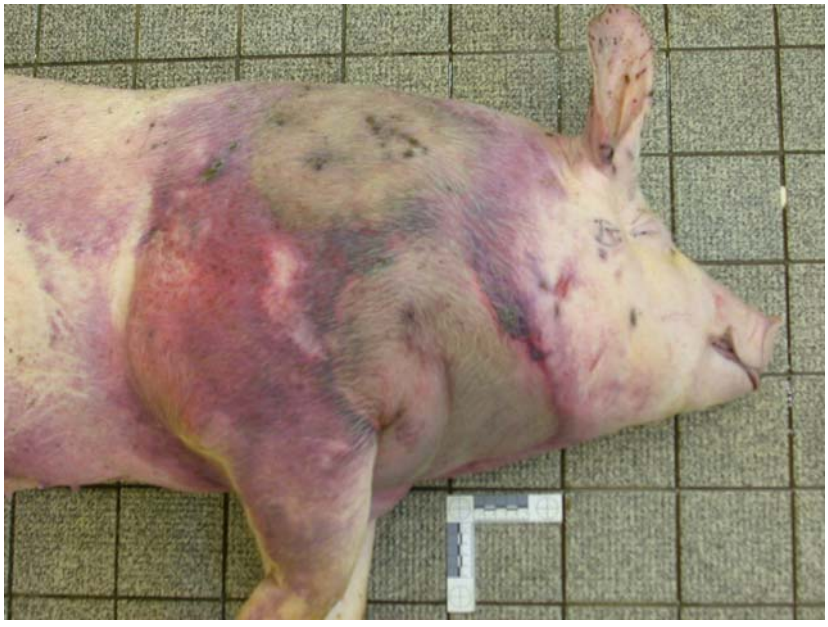

Figure 1: Marked swelling and irregular areas of skin discoloration and hemorrhage associated with *Clostridium septicum* infection.

HORSES

Use of hormone assays to determine the pregnancy status of mares

Kristiina Ruotsalo, Tracey Chenier

Determination of the pregnancy status of a mare may occasionally be problematic, especially in ponies and miniature horses in which the traditional methods of ultrasound examination and palpation of the reproductive tract may not be suitable due to their small stature. The use of hormone assays may be increasingly relied upon. A number of hormone assays are available and are briefly reviewed below.

Equine chorionic gonadotropin (ECG, formerly called pregnant mare serum gonadotropin, PMSG) is produced by the endometrial cups with serum levels rising from approximately 35 days of pregnancy to a peak at 60 - 80 days of pregnancy, followed by a subsequent fall to baseline by 100 - 120 days. Measurement of ECG between 45 - 90 days of pregnancy has been reported to result in an approximate 5% false-positive and a 10% false-negative rate in pregnancy diagnosis. These values may be related to the large individual variation in the ECG profile of mares, or to the fact that the endometrial cups continue to produce ECG until approximately 120 days of gestation even if the fetus dies.

Progesterone is produced by the corpora lutea during the first half of gestation. Measurement of progesterone alone will not confirm that the mare is pregnant, but quantification of this hormone during the phase of corpora lutea dependence may determine if the mare is producing sufficient amounts to maintain the pregnancy. The absolute concentration of progesterone needed to maintain pregnancy remains poorly defined although it has been suggested that concentrations greater than 2.0 ng/mL (6.32 nmol/L) should be present in early pregnancy. Beginning at about 70 days of gestation, the fetoplacental unit produces progestagens, primarily pregnenolone and α-pregnane, as well as estrone sulfate. Progesterone production by the fetoplacental unit is minimal. Progesterone levels of pregnant mares between 200 and 310 days gestation are commonly <6 nmol/L. Unlike other species, from 310 days to term there is a rise in serum progesterone in the mare. Therefore, measurement of progesterone levels after 150 days is not particularly informative in mares since most assays do not quantitate total progestagens. Measurement of estrone sulfate at this stage is more informative.

Estrogens are produced during equine pregnancy first by ovarian luteal tissue, with this source gradually subsiding by approximately day 120 of pregnancy. A second increase in serum estrogens occurs after day 70 of gestation with production of estrogens from the fetoplacental unit commencing. Therefore, evaluation of total estrogens (including estrone sulfate) is considered a reliable indicator of fetal viability with some researchers claiming 99% accuracy at >90 days of pregnancy. Estrogen levels are known to fall dramatically within hours of fetal death.

Obviously, the endocrinology of equine pregnancy is complex and as such there is no single hormone or panel of hormones that will be appropriate for all stages of pregnancy. The use of a tandem hormone profile has been advocated by some researchers. A study involving miniature horse mares suggested that a hormone panel including estrone sulfate and ECG had a 90.1% sensitivity and a 97.5% specificity for the diagnosis of pregnancy when thresholds for estrone sulfate were > 6.0 ng/mL and for ECG >500 ng/mL were set. Further improvements in sensitivity and specificity were obtained when a single value for ECG >300 ng/mL and an increase in estrone sulfate over 45 to 60 days was noted; such results indicated the presence of a viable fetus with 100% sensitivity and specificity. As such, the use of hormone assays, including ECG for mares between days 45-90 of pregnancy, and total estrogens (including estrone sulfate) for mares from 90 days to term should be considered to confirm pregnancy, especially in those animals difficult to manually examine.

No single hormone or panel of hormones is appropriate for all stages of pregnancy.

The AHL currently offers total estrogen and ECG assays through Cornell University and BET labs of Lexington Kentucky with **total estrogens priced at $32 US** and **ECG priced at $40 US**, plus shipping fees. Two mL of serum is required.

Progesterone is determined at the AHL, requires 1 mL of serum, and is priced at **$20.40. The progesterone assay currently employed at the AHL does not cross-react significantly with the progestins produced by the mare in the latter portion of pregnancy, and therefore is best suited for determination of progesterone during early pregnancy.** *AHL*

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Ontario Racing Commission Death Registry: 2003-2006 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. Summaries of necropsy submissions to the Animal Health Laboratory under this program and diagnoses for these cases are provided in the following tables. *AHL*

Table 1. Breed distribution of ORC Death Registry submissions to the AHL, 2003-2006

Breed / Year	Standardbred	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**

Table 2. Necropsy diagnoses of ORC Death Registry submissions by body system, 2003-2006

Diagnosis by body system	2003	2004	2005	2006
Fracture / limbs	53 (42%)	69 (49%)	48 (44%)	42 (39%)
Fracture / other	10	4	7	13
Non-fracture musculoskeletal	8	6	6	8
Gastrointestinal	15	19	17	16
Respiratory	21	17	9	11
Cardiovascular	5	6	5	5
Central nervous system	6	11	7	4
Integumentary	0	0	1	2
Renal	0	2	0	0
Hematopoietic	2	1	1	0
Whole body conditions	1	7	5	2
Cause of death undetermined	4	0	4	4
Total	**125**	**142**	**110**	**107**

Table 3. Limb fracture distribution of ORC Death Registry submissions by anatomic location and breed, 2006

Fracture site	Total	Standardbred	Thoroughbred
P1	13	10	3
RF/LF/RH/LH	5/0/2/6	3/0/2/5	2/0/0/1
P1 and P2 (RF)	1	0	1
P3 (LF)	1	1	0
Mc3	6	0	6
R/L	5/1	0/0	5/1
Mt3 (L)	1	0	1
Mc3 and P1	2	0	2
R/L	1/1	0	1/1
Mc3 and proximal sesamoid (RF)	1	0	1
Carpus	7	0	7
R/L	4/3	0/0	4/3
Proximal sesamoid (LF)	5	4	1
Tibia	2	0	2
R/L	1/1	0/0	1/1
Humerus (L)	2	1	1
Patella (R)	1	1	0
Total	**42**	**17**	**25**

COMPANION ANIMALS

Canine surgical pathology: tumor diagnoses in 2006

Beverly McEwen, Josepha DeLay

Biopsy of suspected tumors should provide the clinician with information needed to effectively manage their patient. This includes the type of tumor, its predicted behavior and whether the resection margins are clear, which indicates complete excision. In addition to routine hematoxylin-and-eosin stained sections, the pathologist may recommend additional special stains or immunohistochemistry to accurately categorize the neoplasm, especially for those with anaplastic features.

In 2006, there were 779 biopsy submissions of tumours from 90 breeds of dogs to AHL histopathology. Most of the cases consisted of single tumors, however, about 1% of the cases had multiple tumors submitted. The most commonly affected breeds were mixed breed dogs (26%), Labrador Retrievers (10%), Golden Retrievers (9%), German Shepherds (4%), Boxers (4%) and Siberian Huskies, Shih Tzus, Rottweilers, Jack Russell Terriers, Cocker Spaniels and Miniature Schnauzers (each at 2%). The average age was 8.25 years (range 77 days to 16 years).

There were 118 distinct types of tumours identified. Happily, about 56% of the tumours were benign, but 12% were locally invasive and 32% were malignant. Tumors were identified from every body system, however, **skin tumors accounted for over half of the cases, followed by mammary gland, oral, bone, eyelid, lymph node, urinary, splenic and intestinal tumors**. *AHL*

Table 1. Most common tumors in canine biopsies, AHL histopathology, 2006

Tumor	% of diagnoses	Most common site (% of diagnoses)	Top 3 breeds affected*
Mast cell tumor (all grades)	10%	Skin (99%)	Labrador Retriever, Boxer, Golden Retriever
Benign cutaneous histiocytoma	7%	Skin (100%)	Boxer, Shih Tzu, Labrador Retriever
Lipoma	6%	Skin (98%)	Labrador Retriever, Standard Schnauzer, Jack Russell Terrier, Golden Retriever**, Border Collie, English Springer Spaniel
Mammary carcinoma	4%	Mammary gland (100%)	Siberian Husky, German Shepherd, Labrador Retriever
Sebaceous gland adenoma	4%	Skin (96%)	Poodle, Shih Tzu, Labrador Retriever
Osteosarcoma	3%	Bone (96%)	Golden Retriever, Labrador Retriever, Rottweiler
Hemangiosarcoma	3%	Spleen (50%), skin (42%)	Golden Retriever, Labrador Retriever, Bull Mastiff
Perianal gland adenoma	3%	Skin (100%)	Siberian Husky, Golden Retriever (other breeds were single occurrences)
Fibromatous epulis of periodontal origin	3%	Oral cavity (100%)	Golden Retriever, Boxer, Labrador Retriever**, Miniature Schnauzer, Eskimo Dog
Trichoblastoma (previously known as basal cell adenoma)	3%	Skin (100%)	Cocker Spaniel (other breeds were single occurrences)
Lymphoma	3%	Lymph nodes (78%)	Standard Poodle, Jack Russell Terrier, German Shepherd, Doberman Pinscher**
Hemangioma	3%	Skin (96%)	Boxer, Golden Retriever (other breeds were single occurrences)
Malignant melanoma	3%	Oral cavity (85%)	Rottweiler, Golden Retriever, Labrador Retriever, Cocker Spaniel**

*excludes mixed breed dogs; **more breeds included if percentage affected was the same.

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