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AHL Newsletter

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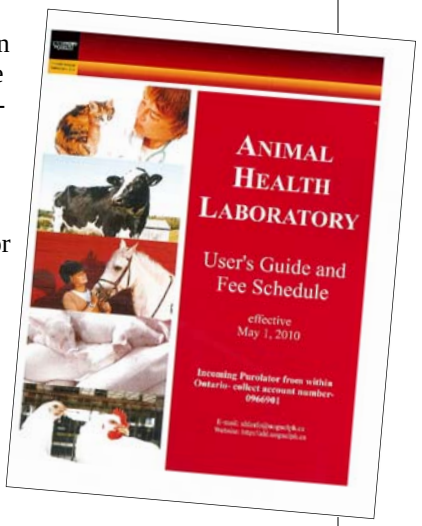
May 1, 2010, AHL User's Guide and Fee Schedule



- Effective May 1, 2010. We would be happy to send clients additional copies.
- Our Fee Schedule is accessible by Vet-clients only - at http://ahl.uoguelph.ca under the Veterinary Menu - click on Client Login and enter your username and password. Here you can access both the Food Animal and Companion Animal Fee Schedule. Please contact us and we'll gladly set you up with an account!
- Updated pricing effective May 1, 2010 to April 30, 2011.
- Expansion of histopathology with the addition of full tumor margin evaluations as well as many new IHC stains.
- New *C. burnetii* ELISA, *C. psittaci* RT PCR, APP PCR, PRRSV ELISA HerdChek X3, swine *Influenza A virus* MultiS-Screen antibody ELISA, IBRV PCR, BRSV PCR, *Bluetongue virus* PCR, equine influenza virus real-time RT-PCR, PRRSV Tetracore Next Generation real-time RT-PCR.
- Fecal egg counts - both McMaster (minimum detection 50 epg) and Wisconsin (minimum detection 1-2 epg) techniques are available.
- The AHL will not be charging HST on lab testing. *AHL*

White-nose syndrome in Ontario bats

Durda Slavic, Ian Barker, Doug Campbell, Cheryl Massey, Lenny Shirose, Melanie Whalen, David Cristo

In March 2010, the Animal Health Laboratory in collaboration with the Canadian Cooperative Wildlife Health Centre (CCWHC) confirmed **the first case of white-nose syndrome (WNS) in bats in Ontario in the Bancroft-Minden area** (http://www.ccwhc.ca/wns_article.php). Subsequent surveillance efforts established the presence of WNS in additional bat hibernacula across Ontario and Quebec.

WNS is an emerging bat disease initially discovered in 2006 in a cave near Albany in New York State. Since then, WNS has continued to decimate bat populations in the northeastern United States. It is estimated that over one million bats have succumbed to the disease to date. The bats suffering from this disease are very thin, have little or no fat stored, and they tend to fly in daylight during the winter. A circle of white powdery fungal growth is usually present on the muzzle, ears and/or wings (Fig. 1). A psychrophilic fungus, ***Geomyces destructans,*** has been isolated consistently from WNS but its role in the disease remains to be established. At present, it is not clear if this organism is the primary cause of the disease or an opportunistic pathogen.

Fig. 1 White-nose (arrows) in a bat.

No human health risks associated with WNS have been reported to date. However, the decrease in bat population due to WNS is a major concern with regards to their many ecological roles, including predation of flying insects. For instance, a single bat can consume nightly, an amount of insects equal to their own body weight. *AHL*

Ten new AHSI projects approved

Jane Gaviller-Fortune, Alicja Zachertowska

A fourth call for proposals went out for **Animal Health Strategic Investment** (AHSI) projects in February 2010, and in March, 10 new projects were approved for funding as well as 2 project extensions. AHSI projects now total 41!

Pjt ID	Project lead; Project team members	Project title	Pjt term
09-01a	Timothy Blackwell, OMAFRA; Franklin Kains, Kathy Zurbrigg	Enhancing disease surveillance in Ontario through a producer-based mortality reporting system	1.5 yr ext.
09-09a	Hugh Cai, AHL; Susy Carman, Josepha DeLay, Murray Hazlett, Beverly McEwen, Durda Slavic	Continuing investigation of infectious etiology of small ruminant abortion in Ontario with emphasis on *Chlamydophila* spp. and *Coxiella burnetii*	1.5 yr ext.
10-01	Janet Alsop, OMAFRA; Suzanne Burlatschenko, Susy Carman, Jim Fairles, D.L. (Hank) Harris, Zvonimir Poljak	Using glycantyping to predict outcome in Ontario sow herds experiencing porcine reproductive and respiratory (PRRSV) outbreaks	2 yr
10-02	Susy Carman, AHL	Validating and implementing new real time PCR tests and ELISAs for mammalian viruses	2 yr
10-03	Shu Chen, AFL; Hugh Cai, Murray Hazlett, Bruce Keown	Molecular typing of *Coxiella burnetii* identified in Ontario by multiple-locus variable number of tandem repeat analysis (MLVA)	2 yr
10-04	Andria Jones, OVC; Jocelyn Jansen, David Kelton, Paula Menzies, Davor Ojkic, Durda Slavic	Johne's disease in Ontario's small ruminant dairy industries: Prevalence, potential risk factors, and performance comparison of serum, milk and fecal diagnostic methods	2.5 yr
10-05	Harold, Kloeze, CFIA; Gaston Annamunthodo, Beverly McEwen, Shamir Nizar Mukhi, Bruce McNab, Andre Vallieres	Expanded data transfer from AHL to CAHSN: In support of improved notifiable disease reporting to CFIA, emergency preparedness, timely disease detection and data analysis	2 yr
10-06	Janet MacInnes, OVC; Shu Chen, Stefan C. Kremer, Zvonimir Poljak, Durda Slavic	Comprehensive evaluation of bacterial hazards in the upper respiratory track of swine by terminal restriction fragment length polymorphism (T-RFLP) analysis	2.5 yr
10-07	Lori Moser, OPIC; Doug MacDougald, Martin Misener, Zvonimir Poljak	Regional PRRS elimination trial	2 yr
10-08	Durda Slavic, Susy Carman, AHL	Summer student assistantships, AHL Mammalian Virology and Bacteriology	0.5 yr
10-09	Maria Spinato, AHL; Jan Shapiro	AHL necropsy laboratories emergency preparedness exercises and incident command system training	1.5 yr
10-10	Patricia Turner, OVC; Marina Brash, Susy Carman, Éva Nagy, Brian Tapscott	Prevalence and characterization of *leporid herpesvirus-4* (LHV-4) in Ontario rabbits, an emerging rabbit pathogen	3 yr

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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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Important changes for companion animal histopathology submissions beginning May 2010

Josepha DeLay, Murray Hazlett

Guidelines and fees for companion animal histology cases submitted to the AHL, including biopsies and in-clinic necropsy samples, changed as of May 1, 2010. Veterinarians and clinic staff should be aware of these changes, which will impact the tests requested by the clinic and client billing, as well as information expected from the histology report (e.g., tumor margin evaluation). These guidelines are printed on the reverse of the AHL companion animal cytology and histology submission form.

1. Number of tissue samples per submission:
Biopsies are considered as up to 2 tissues (or biopsy sites), OR multiple skin, punch, or endoscopic biopsies. Cases for which 3 or more tissues are submitted will be classified as **histopathology, companion animal PM,** and will include samples from in-clinic necropsies as well as cases with biopsies from 3 or more sites. An additional fee-per-slide will be charged for those cases in which more than 10 slides are required to accommodate the submitted tissues.

2. Margin evaluation of tumor excisional biopsies must now be requested as a separate test, in addition to histopathology requested for tumor diagnosis. Margin evaluation will include inking of the intact biopsy and sectioning to capture peripheral surgical margins in 4 quadrants and the deep surgical margin. For those cases where margin evaluation is NOT requested, surgical margins will be assessed on only 1 or 2 sections (if possible) and the pathologist will make a notation if there is tumor at or near margins, however interpretation will be limited. **Specific tumor margin evaluation must be requested at the time of biopsy submission**; requests made after histologic diagnosis of the original biopsy will be subject to additional fees for retrimming and processing.

Please contact us if you have any questions regarding histology submissions. *AHL*

Companion animal histopathology tests	**Test code**	**Sample number / type (fixed tissues)**	*Fee*
Histopathology, biopsy / mail-in	*histcm*	up to 2 tissues or biopsy sites, OR multiple skin, punch, or endoscopic biopsies	$69
Histopathology, tumor margin evaluation	*histt*	in addition to biopsy fee above – includes preparation of 4 additional slides to capture tumor margins – must be requested at time of biopsy submission	$30 per tumor
Histopathology, on companion animal in-house PM or multiple tissues	*histn*	applies to submissions with 3 or more tissues, such as samples from in-clinic necropsies or biopsies from multiple sites	$95 - up to 10 slides; additional slides $5 ea

The importance of referral for necropsy of potential legal cases

Josepha DeLay, Murray Hazlett, Jim Fairles

There has been a recent increase in necropsy submissions to the AHL for which partial post-mortem examination has already been conducted at the referring clinic. Each involved potential legal situations with either animals that died under veterinary care, including anesthetic deaths, or suspected abuse victims. **In cases with potential legal implications, submission of the entire, intact body to a diagnostic laboratory is recommended for necropsy examination by a veterinary pathologist.** A concise but thorough history should be included with the submission, as well as copies of radiographs and results of ante-mortem laboratory tests such as CBC, serum biochemistry, and urinalysis.

Partial dissection and opening of body cavities can destroy or mask important pathologic changes. Also, lesions documented by the referring veterinarian can be impossible to substantiate when a pathologist examines a previously necropsied body, leading to confusion and unnecessary complication of what may already be a contentious case. In suspected cases of abuse, important evidence is frequently lost as a result of post-mortem examination by a non-pathologist. For cases culminating in a court appearance, the pathology experience and credentials of the person performing the necropsy examination are established as part of the legal proceedings and can impact the outcome of the case.

Submission to an independent laboratory for necropsy can indicate to owners that the veterinarian would also like to find out the cause of death, and is a sign of transparency. AHL pathologists do not discuss these cases with owners, but rather only with the referring veterinarian or associates in the same clinic. Exceptions are those cases where release of information is required by law (e.g., animal abuse cases, court subpoena, reportable disease). If an owner submits an animal to the AHL for necropsy, they must name a licensed veterinarian in Ontario as a professional contact to whom the case report is to be released. If the owner is paying for the necropsy, they will receive a final copy of the report, but will be referred to this veterinarian for interpretation of the necropsy report. We do not have a veterinarian-client-patient relationship with the owner, and cannot provide advice directly to them.

If presented with these situations, please feel free to call the laboratory to discuss the case with the pathologist or client services veterinarian regarding procedures and logistics. *AHL*

New real-time PCR tests available at the AHL

Susy Carman

The AHL now offers real-time PCR tests for:

***Influenza A virus*, equine (AE1 and AE2)**

- detects both AE1 and AE2 strains of equine influenza virus, and has a sensitivity of 8 copies per reaction
- identified all 11 strains of equine influenza virus isolated at the AHL over the last 10 years
- specific for *Influenza A virus*, as it does not identify other equine viruses
- submit deep nasal pharyngeal swabs in 0.5 mL of saline from live animals or lung from dead animals
- fee is $38 per test

***Infectious bovine rhinotracheitis virus* (IBRV)/*Bovine herpesvirus 1* (BoHV-1)**

- detects all strains of IBRV, and has a sensitivity of 8 copies per reaction
- identified all 20 strains of IBRV isolated at the AHL over the last 11 years
- specific for IBRV, as it does not identify other bovine viruses
- submit deep nasal, conjunctival or vaginal swab in 0.5 mL of saline from live animals; or trachea, lung, brain of dead animals; or lung, adrenal gland, rumen, liver, kidney, spleen, placenta of fetuses
- fee is $29 per test

***Bovine respiratory syncytial virus* (BRSV)**

- detects all strains of BRSV, and has sensitivity of 8 copies per reaction
- specific for BRSV, as it does not identify other bovine viruses
- submit deep nasal swabs from live animals, or 3 pieces of lung from the dorsal lung lobes from dead animals
- fee is $29 per test.

***Bluetongue virus* (BTV)**

- targets the highly conserved genome segment 1, encoding the viral polymerase VP1
- identifies all 24 serotypes of BTV, and has a sensitivity of 8 copies per reaction
- submit 5 mL of EDTA blood from febrile animals, or lymph node and spleen from dead animals
- fee is $30 per test

For more information on the use of these real-time PCR tests please contact Dr. Susy Carman at 519-824-4120 ext 54551 or at scarman@uoguelph.ca *AHL*

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We look forward to assisting you in all of your animal diagnostic needs.

From the Toxic Garden: Castor bean (*Ricinis communis)* toxicosis

Margaret Stalker, Brent Hoff

The showy castor bean plant is a popular ornamental in Ontario gardens, and is usually sold without any attached warning labels despite containing the compound ricin, a listed bioterrorism agent and one of the most potent natural toxins known. The castor bean is a member of the Euphorbiaceae family, native to tropical Africa, and grows as a 1-4 m tall specimen with large, palmate, green or red leaves, and clusters of seed pods covered with fleshy spines containing 3 smooth, glossy seeds mottled with black, brown and grey spots. **All parts of the plant are poisonous, although the highest concentrations of ricin are present in the seeds and pods.** Various domestic mammals and poultry are susceptible, including humans, although most reports are from dogs. Livestock and horses may be intoxicated by eating garden trimmings. A recent review of castor bean toxicosis in 98 dogs by the ASPCA documented 76% morbidity and 9% mortality.

Castor beans are grown commercially for production of castor oil, used in lubricating oils, paints, varnishes and as an orally administered medicinal purgative. Castor oil is extracted from the plant leaving behind the water soluble toxins ricin and ricinine in the bean pulp. Ricin is a lectin, a glycoprotein that binds and inactivates ribosomes, inhibiting protein synthesis. Ricinine is a low molecular weight piperidine alkaloid toxin found in the seeds and leaves that contributes additional toxicity. The toxins are co-extracted, and analysis of ricinine by LC/MS in stomach content has been used analytically as a biomarker of ricin exposure.

The seeds are attractive to children and inquisitive pets, and if ingested, are non-toxic if the hard, water-impermeable seedcoat remains intact. However, mastication of the seed releases the toxins for absorption. Clinical signs may occur within 24-36 hr, and their severity depends on amount ingested (the estimated oral lethal dose is about 8 seeds for an adult). Poisoning may result in high morbidity, with vomiting, watery to hemorrhagic diarrhea, abdominal pain, progressing to multiorgan failure and death.

Treatment is symptomatic and supportive, as there is no known antidote. *AHL*

Reference

Albretsen JC, et al. Evaluation of castor bean toxicosis in dogs: 98 cases. J Am Anim Hosp Assoc 2000;36:229-233.

Mouser P, et al. Fatal ricin toxicosis in a puppy confirmed by liquid chromatography/mass spectrometry when using ricinine as a marker. J Vet Diag Invest 2007;19:216-220.

Figure 1: Castor bean plant (*Ricinus communis* L.)

Figure 2: Castor bean seeds.

AHL Lab Reports

RUMINANTS

The AHSI small ruminant abortion project – an update

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

We would like to thank all of the producers and veterinarians who have submitted specimens, all of the AHL lab staff – clerical, technical and veterinary - and especially OMAFRA for funding this work via the Animal Health Strategic Investment (AHSI). This is a large project and it could not be done without the help of many people.

This project was started last spring to determine guidelines for interpretation of small ruminant abortion tests, especially for quantitative real-time PCR tests. The project also allows us to evaluate immunohistochemistry tests, and will provide a better understanding of the causes of goat and sheep abortions in Ontario.

The project received additional funding from AHSI to continue for at least another 6 months, and we have analyzed some of the data related to *Coxiella burnetii* and *Chlamydophila abortus* (formerly *Chlamydia psittaci*). We have been testing the samples using *Chlamydophila* spp. PCR and quantitative real-time PCR for *Chlamydophila abortus* and for *Coxiella burnetii*. Other tests include *Chlamydophila* spp. antigen ELISA, bacterial culture isolation, modified acid-fast staining, BVDV PCR, immunohistochemistry (*Coxiella*, *Chlamydophila*, *Toxoplasma*), and pathology examination (gross and histology). The pathology final diagnosis is used as the gold standard to evaluate different tests and the usefulness of different sample types (Figure 1).

As of mid-April, we had completed 104 abortion cases (47 caprine and 57 ovine). For *C. burnetii* in goats, there were 36 cases with positive PCR results, but only 10 abortions attributed to *C. burnetii* by pathology diagnosis. These 10 cases had higher copy numbers of *C. burnetii* in the tissues tested (Table 1). In sheep, there were 46 positive cases with 6 attributed to *C. burnetii.* Similarly, these 10 cases had higher copy numbers of *C. burnetii* in the tissues tested (Table 1). For both *Coxiella burnetii* and *Chlamydophila abortus*, the copy numbers on rt-PCR testing are 100 to 1,000 times greater in placenta than in lung or stomach content (Table 2).

Please continue to submit both placenta and fetus form small ruminant abortions is separate bags. Do not send a fetus without an accompanying placenta. If multiple abortions are submitted, please identify each fetus and its respective placenta. *AHL*

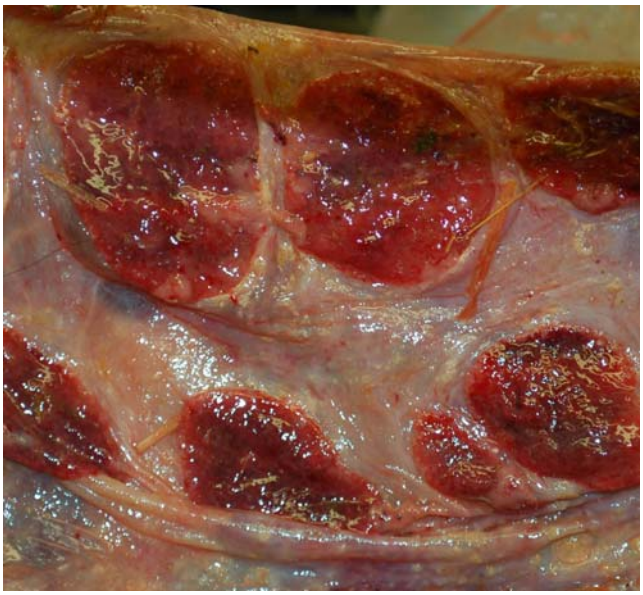
Table 1. *Coxiella burnetii* quantitative rt-PCR results in positive and negative cases of *C. burnetii* abortion as determined by pathology diagnosis.

Caprine	Neg (26)	Pos (10)	**Ovine**	Neg (40)	Pos (6)
Mean	1.07×10^5	8.54×10^9		7.86×10^6	4.79×10^9
Median	6.35×10^2	2.77×10^9		9.16×10^1	4.11×10^8

Table 2. *C. burnetii* and *C. abortus* quantitative real-time PCR results for different sample types for all 104 cases

Specimen	**Placenta**	**Lung**	**Stomach**
Coxiella (mean copies/uL)	1.17×10^9 (n=98)	7.70×10^6 (n=65)	6.23×10^6 (n=28)
*C. abortus (*mean copies/uL)	6.29×10^5 (n=47)	3.88×10^3 (n=36)	1.60×10^4 (n=21)

Figure 1. Placentitis due to *Coxiella burnetii*. Note the severe intercotyledonary thickening and fibrinous exudate on the surface of cotyledons.



SWINE

IDEXX HerdChek PRRSV X3 ELISA now available at the AHL

Susy Carman, Beverly McEwen, Jim Fairles

The new IDEXX *Porcine reproductive and respiratory syndrome virus* (PRRSV) Antibody Test Kit, (HerdChek PRRS X3 antibody ELISA) is now available at the AHL. This new generation IDEXX ELISA **detects IgG antibodies** in serum as early as 8-10 DPI. It detects antibodies directed to the nucleocapsid of both North American and European genotypes of PRRSV, as did the earlier IDEXX HerdChek PRRS 2XR.

As for the previous IDEXX HerdChek PRRS 2XR ELISA, sample/positive (S/P) ratios of less than 0.400 are considered negative for antibody to PRRSV, and S/P ratios greater than 0.400 are considered positive. Titer groups are no longer included.

IDEXX reports the diagnostic sensitivity using 1042 sera collected from field-exposed pigs, PRRSV-vaccinated pigs, and experimentally infected pigs to be 98.8%. They report the diagnostic specificity using 1445 sera from characterized PRRSV-negative pigs to be 99.9%. **An important benefit of this new PRRS X3 ELISA is the enhanced specificity, with a 90% reduction in singleton false-positive reactors.**

We compared the IDEXX HerdChek PRRS X3 antibody ELISA with the earlier IDEXX HerdChek PRRS 2XR antibody ELISA by parallel testing 166 sera. There was excellent agreement, with kappa value 0.89 (0.82, 0.96), which indicates almost perfect agreement.

We also compared the results of 13 single sera using the IFA test and found there was perfect agreement with the IDEXX HerdChek PRRS X3. However, there were 11/13 false positives using the IDEXX HerdChek PRRS 2XR ELISA, illustrating the enhanced specificity and reduction in singleton false-positive reactors.

The fee for each test will be $8.00. *AHL*

Tetracore Next Generation NA/EU PRRSV multiplex real-time RT-PCR is now available at the AHL

Susy Carman, Beverly McEwen, Jim Fairles

The new Tetracore Next Generation NA/EU PRRSV Multiplex real-time RT-PCR is now available at the AHL. This next-generation test detects both North American (NA) and European (EU) genotypes of *Porcine reproductive and respiratory syndrome virus* (PRRSV), as did the earlier first-generation Tetracore NA/EU/LV PRRSV multiplex real-time RT-PCR reagents. However, **this new PCR now differentiates NA and EU genotypes in the same reaction**. Samples are reported as 'NA POSITIVE', 'EU POSITIVE' or 'not detected'. For serum, and other samples where volumes can be measured, the quantity of virus is reported as 'copies/mL'. An internal control is included, so each sample is evaluated for the presence of PCR inhibitors.

This test has broad reactivity and was field validated at the South Dakota State University using 5,348 diagnostic samples (blood swabs, serum, oral fluids, semen), and 350 sequenced samples of various strains with known RFLP type. It was also validated at the OIE PRRSV Reference Lab using 30 widely divergent EU strains, including subtypes 1, 2 and 3. Tetracore reports the analytical sensitivity of this test to be 4 copies/mL. The diagnostic specificity using 30 NA isolates was 99%, with analytical specificity of 100%. The analytical specificity using 27 EU and Lelystad viruses found in the USA was 100%.

At the AHL, we parallel-tested sera from 47 PRRSV-positive animals from known infected herds and 99 PRRSV-negative sera/blood swabs from PRRSV-negative boar studs using both the Tetracore Next Generation NA/EU PRRSV multiplex real-time RT-PCR and the Tetracore first-generation NA/EU/LV multiplex real-time RT-PCR currently in use at the AHL. **We found perfect agreement with kappa value 1.00 (1.00 to 1.00). The relative sensitivity was 100% (95% CI; 92.5,100.0), with relative specificity of 100% (95% CI; 96.3, 100.0).**

This fee for this test will be $28 for tissues and semen, and $25 for serum or blood swabs. *AHL*

IDEXX *Influenza A virus* MultiS-Screen antibody ELISA now available at the AHL

Susy Carman, Beverly McEwen, Jim Fairles

The **IDEXX Avian *Influenza A virus* MultiS-Screen antibody ELISA** is now available at the AHL. This antibody ELISA is a competitive assay and was originally developed for use in avian species. However, since it is a competitive ELISA, it can also be used for other animal species, including swine, to find antibody in sera directed to all strains of *Influenza A virus*.

Results for this competitive *Influenza A virus* antibody ELISA will be reported as "sample to negative (S/N) ratio". A serum with S/N ratio greater than 0.673 is considered to be negative for antibody to *Influenza A virus*. A serum with S/N ratio less than or equal to 0.673 is considered to be positive for antibody to *Influenza A virus*.

Using 453 sera from swine infected both experimentally and naturally with H1N1 (including the pandemic strain of H1N1), H1N2, H2N3, and H3N2 strains of swine influenza virus, and using an S/N ratio 0.673 as the cut point, the USDA has shown this ELISA to have diagnostic sensitivity of 96.6% [95% CI: 92.3, 98.9] and diagnostic specificity of 99.3% [CI: 97.6, 99.9] for the detection of antibody to *Influenza A virus* in swine sera.

The fee is $7.75 per test. For more information about this new test please contact Dr. Susy Carman at 519-824-4120 ext 54551 or scarman@uoguelph.ca. *AHL*

Reference

Ciacci-Zanella J, et al. Detection of anti-influenza A nucleoprotein antibodies in pigs using a commercial influenza epitope-blocking enzyme-linked immunosorbent assay developed for avian species. J Vet Diagn Invest 2010;22:3-9.

New Pathobiology - AHL building

- Combined Pathobiology - Animal Health Laboratory building (building 89)
- Combined federal-provincial funding - $37.3 M federal, $25 M provincial
- Planned completion August, 2010

April, 2010

- Move-in Sept/Oct, 2010
- Official opening early Oct, 2010, tentative.

AVIAN/FUR/EXOTIC SPECIES

Mink diagnoses at the AHL from 2007 to 2009

Emily Martin, Marina Brash, Margaret Stalker, Beverly McEwen, Durda Slavic, Davor Ojkic, Bruce Hunter

The top 3 mink diagnoses at the AHL from 2007 to 2009 include Aleutian disease (AD), bacterial pneumonia, and cystitis/pyelonephritis.

Aleutian disease

AD - the most economically important disease of commercial mink - is caused by a parvovirus. There are multiple viral strains and variable disease severity. The acute and chronic (classical) forms of the disease can cause widespread infection on a ranch leading to decreased female fertility, abortions, increased early kit death, and chronic disease with high mortality in adult mink.

Classical AD is more common in older mink (>3-8 wk old) causing immunosuppression and progressive disease. The persistent viral infection triggers an exaggerated immune response with development of immune complexes that deposit in arteries and glomeruli. Clinical signs include rough coat, decreased appetite, decreased activity, weight loss, uremic ulceration of mouth, tongue and foot pads, ulceration of stomach lining, and black feces from digested blood. On necropsy, there is dehydration, organ enlargement (e.g., spleen, lymph nodes), and kidneys are enlarged, pale, yellow and mottled. Death is usually from renal failure.

In the acute (respiratory) form, naive mink kits develop low levels of immune complexes that deposit in lung alveolar cells leading to respiratory distress and death. Mortality peaks at 10-13 days of age. Kits 3-8 weeks and older or mink kits with maternal antibodies develop classical rather than acute AD.

Chronic AD can cause reduced vaccination response and increased mortality due to increased susceptibility to secondary bacterial infections or stress. **There is no vaccine or treatment available for AD**. Control is by test and elimination of infected animals.

Bacterial pneumonia

Pseudomonas aeruginosa* and *Escherichia coli are the 2 most common causes of bacterial pneumonia identified at the AHL. Both organisms cause acute hemorrhagic pneumonia that appear similar on necropsy and require bacterial culture to differentiate.

Pseudomonas aeruginosa is found in water and soil. Mortality varies from individual mink to large outbreaks (30% mortality) occurring in late summer or fall related to stress. Underlying disease (e.g., AD) can increase susceptibility to and severity of disease. Clinically there is depression, labored breathing and hemorrhagic exudate from the nose. On post mortem examination, there is a necrotizing hemorrhagic pneumonia. A vaccine is available.

E. coli pneumonia usually affects individual animals rather than resulting in herd outbreaks. Mortality is usually low and without pattern. AD can cause increased severity of disease and herd outbreaks. The source of *E. coli* is often not identified.

Cystitis, urolithiasis and pyelonephritis

Urinary tract infections due to various bacteria (*Staphylococcus aureus*, *S. intermedius*, *Streptococcus sp.*, *E. coli*, *Proteus mirabilis* or *Micrococcus sp.*) can predispose mink to the development of uroliths. Urinary infections are most common in females around parturition (April-June) and in the summer. Males experience this condition in the summer and fall. Diagnosis is usually after death. On post mortem examination the bladder is usually distended and hemorrhagic containing blood and calculi. The kidneys may also be affected by ascending infections or pyelonephritis resulting in either swollen, pale kidneys or kidneys with a roughened surface. Bacterial culture can support treatment selection.

Diagnostic testing

We provide full service diagnostic services for mink at the AHL, including necropsy, histopathology, bacterial culture, ADV CIE (counterimmunoelectrophoresis) herd testing, and ADV PCR testing and genotyping. For specific instructions on how to submit for AD herd testing, please refer to AHL "LabNote 8: Aleutian mink disease virus testing at the AHL" at: http://www.labservices.uoguelph.ca/labserv/units/ahl/documents/LabNote08-ADV.pdf *AHL*

References

Aleutian Disease in Mink. OMAFRA Fact Sheet. http://www.omafra.gov.on.ca/english/livestock/alternat/facts/97-033.htm

Hunter DB. Aleutian Disease Diagnosis and Control: A Quick Review. Ontario Veterinary College, University of Guelph.

Hunter DB, Lemieux N., ed. Mink…biology, health and disease. Graphic and Print Services, University of Guelph, 1996.

HORSES

Outbreak of equine respiratory disease on an eastern Ontario horse farm

Nonie Smart, Susy Carman

Equine respiratory viruses circulate regularly within horse populations and can be a cause of economic loss in commercial horse operations, where loss of business, as well as disease related diagnostic and treatment costs, adds to the economic impact. **We report an outbreak of equine respiratory disease as it occurred at a small boarding and riding stable, which resulted in closure and quarantine for 4 wk.** The outbreak began 3 wk after routine closure for Christmas holidays. The source of the infection was not determined. However a veterinarian had visited the farm in mid-December to examine one horse, and the stable owner had visited another horse farm at the same time. Neither the stable owner, nor the owners of 2 boarded horses, had any additional contact with other horses. It was most likely that the viruses were brought to the farm via fomites, such as on clothing and footwear, since no respiratory disease was reported by neighboring horse farms. Eight of the 11 horses had received a single equine influenza vaccine in the spring of 2009, while one horse (Gypsy) had received an additional booster vaccine in the fall of 2009.

The farm housed the 11 horses in 4 separate outdoor paddocks, in small groups, with access to shelters. Horses were stabled only during inclement weather or illness. They were fed and handled by one caretaker, making it difficult to effectively isolate sick animals. Nine of 11 horses were likely already exposed to a sick/shedding horse prior to recognition of the outbreak.

The outbreak spread gradually, with 2 horses (Elmo, Skip) mildly lethargic during a training session. They developed pyrexia, mild coughing, mild anorexia and nasal discharge 2 d later and were stabled. Subsequently all riding and training ceased, with all horses examined twice a day for evidence of clinical disease. At no time during the outbreak were clinically normal horses brought into the horse stable where sick horses were housed. Approximately 1wk after the initial cases, 2 additional horses were observed to be mildly lethargic. One horse (Stormy) developed nasal discharge and coughing, which worsened over 5 d, while another (Shorty) exhibited mild pyrexia, coughing and lethargy for approximately 3 d, after which time these clinical signs quickly resolved. The 7 other horses remained clinically normal throughout the outbreak. (continued on p. 19)

Table 1. Acute and convalescent antibody titers to equine influenza-2 virus (EIV-AE2 , H3N8) and *Equine rhinitis A virus* (ERAV)

Paddock group	Age (yr)	Clinical disease[a]	EIV-AE2,H3N8[b] acute/conv ab titers	EIV vaccination in previous 6 mo	ERAV [c] acute/conv ab titers
Beside horse stable					
Elmo	10	C, P, A, N	1:256/1:64	-	1:96/1:64
Skip	10	C, P, A, N	1:64/1:512[d]	-	1:1024/1:1024[e]
JR	22	-	1:32/1:128[d]	-	1:8/1:8
Kiz	20	-	1:32/1:32	-	1:192/1:192
Other side of horse stable					
Rusty	19	-	1:8/1:128[d]	-	1:384/1:128
Gypsy	12	-	1:16/1:16	+	1:1024/1:1536[e]
45 m from horse stable					
Stormy	12	C, N	1:8/1:256[d]	-	1:768/1:192
Shorty	4	N, A	1:16/1:16	-	1:384/1:256
Charm	5	-	<1:8/1:64[d]	-	1:768/1:1024[e]
60 m from horse stable [f]					
Ricky	19	-	<1:8/<1:8	-	1:1024/1:2048[d]
Ram	26	-	ND	-	ND

[a] Clinical disease: C = cough, P = mild pyrexia, A = anorexia, N = nasal discharge
[b] EIV – AE2, H3N8 = equine influenza A2 virus (H3N8), acute and convalescent antibody titers – seroconversion or significant 4-fold or greater increase in antibody titers
[c] ERAV = *Equine rhinitis A virus* acute and convalescent antibody titers
[d] high antibody titers to EIV-AE2,H3N8, implying recent infection
[e] high antibody titers to ERAV, implying recent infection
[f] no direct contact with sick horses

Equine respiratory outbreak - continued from p. 18

Acute sera were collected 1 wk after the first 2 horses became ill, with convalescent sera collected 3 wk later. Initially paired sera from only 4 horses were submitted to the AHL for determination of antibody titers to *Equid herpesvirus-1/4, Equid herpesvirus-2,* equine influenza virus-AE1 (H7N7), equine influenza virus-AE2 (EIV-AE2, H3N8), *Equine rhinitis A virus* (ERAV) and *Equine rhinitis B virus* . Sera of 6 additional horses were later evaluated for antibody titers against only 2 viruses (EIV-AE2, H3N8 and ERAV), identified as being most likely associated with the outbreak, as determined by the initial screening.

Distribution of horses on the farm and acute and convalescent antibody titers for a EIV-AE2, H3N8 and ERAV are shown in Table 1. One horse (Charm) showed seroconversion (negative <1:8 to positive 1:64) and 4 horses (Skip, JR, Rusty, Stormy) showed a significant 4-fold (or greater) increase in antibody titers for EIV-AE2 H3N8, which **implies a serological diagnosis of recent EIV-AE2, H3N8 infection**. One clinically affected horse (Elmo) had a high acute antibody titer (1:256) to EIV-AE2, H3N8, also suggesting recent infection with this virus for 6 horses. Despite the likelihood that all animals were exposed to sick animals and/or transfer of viral agents by fomites (hay or clothing), 4 horses (Kiz, Gypsy, Shorty, Ricky) did not have a change in antibody titers to either EIV-AE2 H3N8 or ERAV, yet Shorty had anorexia and nasal discharge. The earlier vaccination in spring of 2009 with equine influenza virus vaccine may have influenced the change in antibody titers for this clinically affected horse. Not all horses that had a significant increase in antibody to EIV-AE2, H3N8 were observed to be sick (JR, Rusty, Charm), implying subclinical infection occurred in 3 horses. Four horses (Skip, Gypsy, Charm, Ricky) also had high antibody titers (1:1024) in either acute or convalescent sera to ERAV, suggesting recent concurrent infection with this virus. Of these 4 horses, only 1 (Skip) also had clinical disease. However Skip also had a significant increase in antibody to EIV-AE2, H3N8 over the study period.

Clinical signs for all 4 sick horses resolved within 4 wk, although another 4 wk were required to bring the first 2 horses back into full training. These 2 horses had exhibited the most severe respiratory difficulty (tachypnea, wheezing, abdominal lift) for 1 wk, which was likely exacerbated by a history of pre-existing COPD in these 2 horses. *AHL*

Myopathy associated with selenium and vitamin E deficiencies in mature horses and a donkey

Brian Binnington, Brent Hoff, Beverly McEwen, Norma de Rose

Nutritional myopathy usually occurs in foals less than a year of age and it has been associated with deficient tissue and blood levels of selenium and vitamin E. Myopathy with involvement of skeletal, cardiac and masticator muscles is infrequent in mature horses and the association with selenium and vitamin E deficiencies is more variable with deficient or adequate tissue and/or blood levels reported.

At the AHL from January 2009 to March 2010 there were 7 cases of myopathy in mature horses and 1 donkey associated with selenium and vitamin E deficiency. Two horses and the donkey were necropsied. Tissues for histopathology from 1 horse and serum and/or EDTA blood from 5 horses were also submitted. Seven cases occurred in the winter or early spring. Clinical histories recorded stiff gait and paresis (n= 5), poor body condition (n= 4), listlessness (n= 3), dark wine-colored urine (n= 2), and dysphagia with swollen masseter muscles (n= 2). In 4 cases, the diet was reported to be locally grown hay without supplement feeding. Histopathology was conducted in these cases and there was severe myodegeneration characterized by myofiber sarcoplasm hypereosinophilia, hyalinization, granulation, fragmentation, vacuolation, fine mineralization and phagocytosis. Multiple skeletal muscles were affected in the 4 cases, heart in 2 cases, masseter muscles in 3 cases as well as tongue, esophagus, diaphragm and intercostal muscles.

One horse had elevated serum aspartate aminotransferase (AST) of 13,849 U/L (ref. 259-595 U/L) and creatine kinase (CK) of 14,889 U/L (ref. 108-430 U/L). Two additional horses had either elevated AST (64,660 U/L) or CK (1,793,020 U/L) levels.

Low selenium levels were present in serum samples in 3 horses at 0.02, 0.021 and 0.025 µg/mL (ref. 0.12-0.18 µg/mL) or EDTA blood samples in 2 horses at 0.055 and 0.11 µg/mL (ref. 0.17-0.25 µg/mL). Selenium levels in liver were low in 2 horses at 0.047 and 0.082 µg/g (ref. 0.3-1.0 µg/g). Serum vitamin E levels were low in 3 horses at 1.2, 2.4 and 4.5 µmol/L (ref. 4.6-23.0 µmol/L). The donkey had low liver selenium at 0.24 µg/g (ref. 0.3-1.0 µg/g) but the liver vitamin E was adequate at 65.2 µg/g (ref. 20-40 µg/g).

Selenium deficiency diseases occur in Ontario livestock due to low selenium levels in the soil. Hay and other crops in northern Ontario have been found to have variable but low levels of selenium and vitamin E. The myopathy in these horses and donkeys was considered to be associated with deficiencies of selenium and vitamin E. Myopathy due to ionophore toxicosis were considered unlikely as these horses were being fed hay without supplements and the stomach content of the donkey tested negative for the ionophores monensin, narasin and salinomycin. *AHL*

COMPANION ANIMALS

Anesthetic deaths in dogs and cats: lesions associated with resuscitation efforts

Jan Shapiro, Maria Spinato, Beverly McEwen, Tom McLean

A 10-year retrospective study of dogs and cats submitted to the Animal Health Laboratory in Guelph and Kemptville for necropsy to investigate the cause of death during anesthesia was done. The purpose of the study was to identify gross and histological lesions in animals for which routine resuscitation procedures such as positive-pressure ventilation, external cardiac massage, chest compression, tracheal intubation, epinephrine, lidocaine and/or atropine injection by intravenous, intratracheal and/or intracardiac routes, and/or oxygen administration were attempted prior to the animal dying or being euthanized.

Thirty-one dogs and 35 cats fit the criteria of the study, as follows:

- no significant underlying disease or illness was identified in the animals either prior to anesthesia or during a complete necropsy
- dogs were anesthetized for routine neutering, dentistry and/or tattooing
- cats were anesthetized for routine neutering, dentistry and/or declawing

The interpretation of the pathologist was that the necropsy lesions in the cases selected for this report were induced by trauma or compression during resuscitation. These were: pneumothorax with or without obvious perforation of the pleura, severe pulmonary atelectasis, severe lung hemorrhage, frank blood in the thorax or abdomen unrelated to the surgical procedure, frank blood in the trachea, torn liver capsule and disrupted parenchyma, severe thymic hemorrhage and fractured ribs (Table 1). However, it is recognized that pneumothorax, pleural tears, pulmonary atelectasis and tracheal hemorrhage could also be caused by improper ventilation technique during anesthesia or intubation.

Four (11%) cats and 7 (23%) dogs had no lesions. Fourteen (40%) cats and 13 (42%) dogs had multiple lesions. For example, severe atelectasis often accompanied pleural tears or pneumothorax, and hemoperitoneum was associated with tearing of the liver capsule.

Table 1. Lesions found at necropsy of cats and dogs and believed to be caused by resuscitation efforts

Lesion type	# of times lesion seen in CATS	# of times lesion seen in DOGS
Fractured ribs	1	0
Hemoperitoneum	2	4
Hemothorax	5	1
Pneumothorax/ pleural tears	12	3
Pulmonary atelectasis	20	4
Pulmonary hemorrhage	2	1
Tracheal hemorrhage	3	2
Liver trauma	2	1
Severe thymic hemorrhage	0	2 (7 mo, 18 mo old)
Total	**47**	**18**

The higher incidence of post-resuscitation lesions in cats may reflect the difficulty in applying manual pressure and judging ventilation pressure for these small animals, most of which were kittens. Alternatively, it may reflect some unique physiological features of cats. In some human studies, rib and sternal fractures are reported as common complications of CPR. The relatively low incidence of fractures in this case group may reflect increased compliance of these structures in young animals relative to human adults.

Presumably, there is a population of dogs and cats that experience cardiac and/or pulmonary arrest during anesthesia for which resuscitation is successful. It is interesting to note that since many of the individual lesions described above are not necessarily fatal, they may contribute to subtle or unexplained post-anesthetic clinical abnormalities. *AHL*

New test: We offer a new companion animal Bacteriology follow-up test to monitor antimicrobial therapy (cultn2, $23.50, no susceptibilities included, sample submitted from the same animal within 2 mo of first submission *AHL*

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