University of Guelph
Changing Lives Improving Life
Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

Canada Post Publications number - 40064673

Volume 16, Number 2, page 13 **June, 2012** ISSN 1481-7179

In this issue:

AHL Clinical Pathology Laboratory

Back row (left to right): Laura Schilbe, Amparo Prieto, Dr. Brent Hoff, Helen Kocmarek (laboratory technical supervisor). Middle row: Dr. Kris Ruotsalo, Amal Ahmed, Billie Jo Barbosa. Front row: Gwen Jarrett, Cynthia Hong, Sandra Beltran

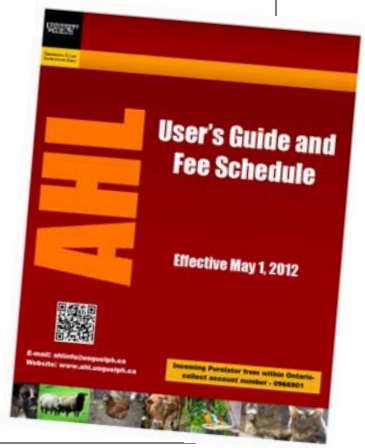
The clinical pathology lab offers a complete range of routine diagnostic testing including hematology, serum biochemistry, endocrinology, coagulation, protein electrophoresis, urinalysis, and cytology. In addition to clinical samples, we also provide customized analysis of samples for researchers. The laboratory employs 8 Medical Laboratory Technologists who have extensive experience with automated instrumentation and multispecies samples. Two clinical pathologists are available for evaluation of cytological specimens and case consultations.

Clinical Pathology Laboratory Update

- A new, fully automated coagulation instrument, the **STA Compact**, has been installed in the laboratory. This instrument will provide rapid, reliable, routine coagulation testing results and will allow future expansion of our coagulation test menu.
- A new **radioimmunoassay for insulin** is currently under evaluation. In addition to equine insulin submissions, we hope to be able to utilize this assay for multispecies analysis.
- A new serum **progesterone assay** is also currently undergoing evaluation.
- Serum **testosterone** testing is being **discontinued** within the laboratory. *AHL*

May 1, 2012, AHL User's Guide and Fee Schedule

- Mailed mid-April - we would be happy to send clients additional copies.
- View and search available tests at http://www.ahl.uoguelph.ca—click on Available Tests.
- To login for fees, for Vet-clients only - at http://www.ahl.uoguelph.ca Contact Josie at AHL ext. 54320 or ahlinfo@uoguelph.ca for the **Client Access** username and password.
- Access to online results is also available – please contact us for further details ahlinfo@uoguelph.ca
- Updated pricing effective May 1, 2012 to April 30, 2013.
- Bacteriology testing update: Mastitis, milk culture, mast-a discount of $0.50 per sample is now available when submitting more than 50 samples for testing from the same herd. *AHL*



Communications at the AHL

Jim Fairles, Client Services Veterinarian, 519-824-4120 ext 54611, jfairles@uoguelph.ca

The AHL strives to communicate with its clients in the most expeditious and timely manner possible.

Telephone: AHL Specimen Reception in Guelph monitors our incoming phone line - **519-824-4120 ext 54530** - from 8:30AM to 6:00PM Monday to Friday and 9:00 AM to 5:00 PM on Saturday/Sunday. We strive to answer the phone but, in high call-volume periods, you may need to leave a message and we will return the call as soon as possible.

The AHL-Kemptville phone is **613 258-8320** - answered Monday to Friday 8:15 am - 12:00 pm, and 1:00 pm - 4:30 pm.

There are several electronic ways to obtain information about submissions, tests, and cases.

- **Our website:** www.ahl.uoguelph.ca There is a considerable amount of information on our website including, submission guidelines, submission forms, LabNotes, and newsletters. Our **User's Guide** and completely searchable **Fee Schedule** are on-line as well. Our veterinary clients need a user name and password to access the User's Guide and Fee Schedule. Please email us at ahlinfo@uoguelph.ca to obtain your user name and password.
- **Info Email** AHL monitors our info email ahlinfo@uoguelph.ca during regular business hours, and can provide timely feedback and information on a wide variety of subjects.
- **Reporting:** All reports can be faxed and/or emailed to clients. With our LIMS, we are also able to use templates that add multiple report To:s, making our ability to report results quite flexible. Please note that currently CFIA EIA reports cannot be reported electronically. These reports must be faxed, couriered or mailed. Please advise us if these results need to be expedited.
- **On-line access to results:** AHL can provide confidential on-line access to client results through our LIMS. The URL for this is http://sapphire.lsd.uoguelph.ca:8080/labservices/logon.jsp Please email ahlinfo@uoguelph.ca to sign up and receive your user name and password (this is an individual password different from the access to the Fee Schedule). On-line access provides easy access to client results.
- **Publications:** LabNotes, Newsletters and other publications are available on our website.

We would be glad to receive feedback on any of our communications methods. Please feel free to email us at ahlinfo@uoguelph.ca or call 519-824-4120 ext 54530.

AHL Newsletter
June, 2012 - Volume 16, Number 2

Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistants: **Helen Oliver, April Nejedly**

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.

Mailing address & contact information:
(**please return all undeliverable Canadian addresses to**:)
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
Email: *holiver@uoguelph.ca*

ISSN 1481-7179
Canada Post Publications number - 40064673

Contributors to this issue:
From Laboratory Services / Animal Health Laboratory:
Mioara Antochi, MD
Marina Brash, DVM, DVSc, Diplomate ACVP
Andrew Brooks, DVM PhD, Diplomate ACVP
Susy Carman, DVM, Dip SA Med, PhD
Jim Fairles, DVM, MBA
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Brent Hoff, DVM, DVSc, DipTox
Emily Martin, DVM, MSc, Diplomate ACPV
Beverly McEwen, DVM, MSc, PhD, Diplomate ACVP
Davor Ojkic, DVM, PhD
Nick Schrier, MSc
Jan Shapiro, DVM, DipPath, DipEqSurg
Durda Slavic, DVM, PhD
Margaret Stalker, DVM, PhD, Diplomate ACVP
Other contributors:
Soren Alexandersen, DVM, PhD, DVSc, **Zhidong Zhang**, DVM, PhD; National Centres for Animal Disease (NCAD), NCFAD-Winnipeg, MB
Maya Andonova; **Mike Drebot,** PhD, Public Health Agency of Canada, Winnipeg, MB
Jocelyn Jansen, BSc, DVM, DVSc, OMAFRA, Elora ON
Paula Menzies - DVM, Dip ECSRHM, MPVM; **Jeff Rau,** DVM, BSc, MSc; **Lisa Santry**, BSc; **Sarah Wootton** PhD; **Denise Yates**, DVM student; OVC
Carole Simard DVM, MSc, PhD, CFIA, St. Hyacinthe, QC
Kevin Vilaca, DVM, MSc, Listowel, ON
Alex Weisz, DVM, Guelph, ON

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.

AHL Lab Reports

RUMINANTS

Cache Valley virus identified as a cause of malformed lambs in Ontario

Jan Shapiro, Andrew Brooks, Paula Menzies, Jeff Rau, Mike Drebot, Maya Andonova, Zhidong Zhang, Soren Alexandersen, Susy Carman, ,

Cache Valley virus (CVV) is an arthropod-borne agent, one of 33 viruses belonging to the *Bunyamwera* serogroup of the *Bunyavirus* genus. The virus is found throughout Canada, the United States and Mexico, and has been isolated from several species of mosquitoes, including *Culex, Aedes, Psorophora, Anopheles* and *Coquillettidia*. While CVV infects a wide variety of mammals, including sheep, goats, cattle, horses, pigs, deer and humans, clinical disease occurs primarily in sheep. Most natural infections in non-pregnant sheep are subclinical. However, in pregnant ewes, the virus may cross the placenta and infect the fetus, with the outcome of fetal infection depending on the timing of *in utero* exposure. Experimental infection of fetuses before 32 d gestation often resulted in early embryonic death, while fetuses infected between 32-48 d had malformations of the musculoskeletal and central nervous systems. In experimental and natural infections, malformations of the brain can include hydranencephaly, hydrocephalus, porencephaly, microencephaly, cerebellar and cerebral hypoplasia and micromyelia. Musculoskeletal lesions include arthrogryposis, scoliosis, torticollis and muscle hypoplasia.

In mid-December 2011 and early January 2012, 2 stillborn lambs from flock A and 1 from flock B, respectively, were submitted for necropsy to AHL-Kemptville. Both flocks were located in eastern Ontario. Flock A reported that 4 of 20 lambs had been born small, with "humpy" withers and stiff limbs, and that there had been an abnormal number of breech births. Often, only 1 of multiple lambs from the same ewe was affected. Flock B reported increased reproductive losses and submitted 1 affected lamb from 50 ewes. **Numerous deformities were found in these 3 lambs, including hypoplasia and dysplasia of the cerebellum, cerebrum and spinal cord, hydrocephalus, arthrogryposis, torticollis, scoliosis, kyphosis, cranial and cervical vertebral malformation, and skeletal muscle hypoplasia.**

Serum from 5 ewes from flock A, including the 2 dams of the 2 deformed lambs submitted for autopsy, and serum from the dam from flock B was tested and found negative for virus neutralizing antibodies to *Bovine viral diarrhea virus* (BVDV) */Border disease virus* (BDV). Fetal tissues from the 3 lambs from both flocks A and B were negative for BVDV/BDV using immunohistochemistry and virus isolation. However, **serum from all 5 ewes from flock A and the serum from the dam of the deformed lamb in flock B were positive for antibody to CVV using a plaque reduction neutralization assay performed at the National Microbiology Laboratory, Public Health Agency of Canada (PHAC), Winnipeg.** Titers ranged from 1:80 to 1:160. RT-PCR testing of tissues from all 3 lambs from flocks A and B was done at the same laboratory. One fetus from flock A tested positive for CVV RNA by RT-PCR and the virus was isolated in Vero cells. Tissues from the second flock A fetus also gave positive CVV RT-PCR results. **Sequence analysis of CVV amplicons identified a genetic variant of CVV distinct from other genotypes currently in GenBank and PHAC databases.** Tissues from the 2 fetuses from flock A were also tested for *Schmallenberg virus* (SBV) using real-time RT-PCR at the National Centre for Foreign Animal Disease (CFIA), Winnipeg, and all found to be negative. Sera from all 6 ewes from both flocks A and B were negative for antibody to SBV using virus neutralization tests.

A third flock (flock C) located in central Ontario was also affected. In early January 2012, 3 deformed lambs were born to 2 ewes. Two lambs were stillborn and 1 lived for 1 week. Sera from the 2 dams were submitted to the AHL in Guelph and were forwarded to the Texas A&M Diagnostic Lab for testing for antibody to CVV using a virus neutralization assay. One serum had a positive antibody titer of 1:128 to CVV, strongly suggesting that this virus was the cause of deformities in this flock.

Anecdotally, CVV has been associated with reproductive loss in sheep in Ontario for many years. However, to the best of our knowledge, **these are the first cases of malformed lambs in Canada in which CVV infection was confirmed by serology of the dam, positive RT-PCR results from lamb tissues, and an isolate grown from fetal tissue**. In Ontario, infection of ewes likely occurs in early pregnancy when ewes are on pasture and mosquito populations are high. This would explain the timing of abortion of malformed fetuses and stillborn lambs in late December through January.

Malformed fetuses can be carried to term, but the ewe may experience dystocia due to the fetal malformations. Ewes that are seropositive to CVV are protected from subsequent infections with CVV, but not other bunyaviruses such as La Crosse virus, San Angelo virus and Main drain virus, which can also cause fetal death, arthrogryposis, hydrocephalus and skeletal deformities. CVV is a zoonotic agent which has been documented to cause febrile and neuroinvasive disease in humans. Patients become infected as the result of being bitten by infected mosquitoes, but not from direct contact with sheep. *AHL*

Maedi-visna virus (MVV) antibody ELISA is now available at the AHL for the detection of MVV antibody in sheep sera and use in MVV control programs

Susy Carman, Beverly McEwen, Paula Menzies, Sarah Wootton, Mioara Antochi, Carole Simard, Jocelyn Jansen, Lisa Santry, Denise Yates

Maedi-visna (MV) is a common and economically important infection in Ontario sheep flocks, and causes progressive pneumonia, weight loss, and decreased milk production in adult sheep. **The HYPHEN BioMed Elitest MVV indirect ELISA is now being used at the AHL to identify antibody to MVV in sheep sera**. Results for the Elitest are reported as positive, suspicious, or negative. For confirmation of low numbers of positive and suspicious individual test results in flocks expected to be negative, we suggest retesting these same sera using the **IDEXX Chekit MVV confirmation antibody ELISA**. The cost for each ELISA is $9.00.

The Hyphen BioMed Elitest MVV ELISA is now being used in the Ontario Maedi-Visna Flock Status program offered by the Ontario Sheep Marketing Agency. This program allows veterinarians and producers to test and eradicate MVV from flocks and then to maintain a low risk status. For more information on this program and its protocols, please contact OSMA at (519) 836-3510 or visit their webpage at www.ontariosheep.org.

For more information on the Elitest MVV, please contact Dr. Susy Carman at 519-824-4120 ext 54551 or scarman@uoguelph.ca *AHL*

Tetracycline susceptibility of *Campylobacter* isolates causing ovine abortions in Ontario

Durda Slavic, Beverly McEwen, Paula Menzies

Historically, *Campylobacter fetus* subsp. *fetus* was considered the main cause of *Campylobacter*-associated ovine abortions. Recently, however, a shift towards a higher isolation rate of *C. jejuni* from ovine abortion has been reported primarily in the United States and New Zealand. It appears that this increased incidence of *C. jejuni*-associated ovine abortions in the United States can be partially attributed to an emergence of a single tetracycline-resistant *C. jejuni* clone.

While investigating the role of *Chlamydophila* spp. and *Coxiella burnetii* in caprine and ovine abortion under an Animal Health Strategic Investment (AHSI) grant, an increased number of ovine abortion submissions was received by the AHL. As *C. fetus* subsp. *fetus* and *C. jejuni* were isolated from some of these cases, susceptibility testing was performed using a microbroth dilution method to determine minimal inhibitory concentration (MIC). MIC was determined primarily for chlortetracycline and oxytetracycline as these are the 2 main drugs that are used for prevention of both *Campylobacter*- and *Chlamydophila*- associated abortions in sheep. In total, MIC was done on 20 *Campylobacter* spp. isolates from Ontario. Seven of these isolates were further speciated as *C. fetus* subsp. *fetus* and 12 as *C. jejuni*. MIC values for chlortetracycline for *C. fetus* subsp. *fetus* isolates were either 1 µg/mL (6 isolates) or 2 µg/mL (1 isolate). Similar MIC values, ≤0.5 µg/mL (2 isolates) and 1 µg/mL (5 isolates), were recorded for oxytetracycline. In contrast, all *C. jejuni* isolates have MIC >4 µg/mL for both chlortetracycline and oxytetracycline, indicating that they are either intermediately susceptible or resistant to both drugs.

Based on these findings, **it appears that tetracycline-resistant *C. jejuni* isolates may be present in Ontario sheep flocks**. As a result, it is very important for small ruminant practitioners when suspecting *Campylobacter*-associated abortions to confirm which species of *Campylobacter* is present before attempting any prophylactic or metaphylactic treatments with tetracyclines. If *C. jejuni* is suspected, antimicrobial susceptibility testing should be done to ascertain which antimicrobial would be effective in its control. A bivalent vaccine for prevention of *C. fetus* subsp. *fetus* is currently available in Canada, and can be used to prevent further abortions due to this disease agent. **The use of tetracyclines in the feed or to mass medicate the ewe flock for the control of infectious abortion (e.g., if abortion due to *Chlamydophila abortus* is suspected) may actually increase the incidence of tetracycline-resistant *C. jejuni* abortions.** For this reason, diagnostic investigation of ovine abortions should always be pursued to confirm the etiology and thus appropriate control measures.

The further investigation of these cases was made possible by AHSI surveillance funding. *AHL*

SWINE

TEGO swine oral fluids collection kit

Susy Carman

TEGO now offers a complete kit for the collection of oral fluids from swine (Figure 1). Each kit includes a bleach free white cotton rope assembled with a nylon cord to suspend the cotton rope, 2 pairs of latex-free gloves, 1 large zip-top plastic bag with tear notch to squeeze and collect the saliva from the cotton rope, one 50 mL centrifuge tube with label and bar code, 1 small zip-top bag for the tube, 1 double-pouch bag for submission of tube and submission form, and instructions for use. Kits (product code A100930) can be purchased from ITL Animal Healthcare, sales@itlanimalhealthcare.com (tel 703-435-6700, fax 703-435-6717). *AHL*

Figure 1. TEGO kit for collection of oral fluids.

Vertebral fractures due to lightning strike in market-weight pigs

Murray Hazlett, Kevin Vilaca

Three 80–100 kg pigs were submitted to the AHL with a history of lameness and paralysis. These pigs were part of a 1,000 head finisher group. Three days previously, there had been a lightning strike on the barn, immediately following which many pigs showed clinical signs of injury. Many pigs were bright and alert, sitting with hindlimbs out to the side, however clinical presentations varied. The most obviously affected pigs were submitted for PM. Some pigs showed clinical signs immediately, while some had a more delayed onset. A total of 36 pigs were affected.

Necropsy of 5 pigs (2 on farm, 3 at the AHL) showed hemorrhage in the gluteal and peripelvic muscles, with markedly enlarged, often hemorrhagic urinary bladders (4 pigs) and a ruptured bladder with uroperitoneum (1 pig). Spinal columns were removed from the 3 pigs submitted to the AHL. In all 3 pigs, **there were fractures of the 6th or 7th lumbar vertebrae, with fracture also of the sacrum in 1 pig.** No burn marks were seen.

Significant histologic lesions consisted of hemorrhage in muscles and surrounding the spinal cord. In the lumbar spinal cord, swollen axons were seen in 1 pig, and there was fibroblast activation in meninges.

The clinical presentation and duration of injury are compatible with the lesions seen. Traumatic lesions of the sacral cord and lumbar nerve roots within the vertebral canal can result in flaccid (lower motor neuron) paresis or paralysis of the pelvic limbs and an atonic bladder, resulting in distension and overflow incontinence.

Fractures of the caudal lumbar vertebra or first sacral vertebral segment associated with electrocution, including lightning, usually involve large pigs, and have been described in several publications. These fractures are typically longitudinal (Figure 1), likely caused by severe rapid contraction of the powerful extensor muscles of the hip in heavily muscled pigs. *AHL*

Reference

Sanford SE, Josephson GKA. Vertebral fracture and posterior paralysis in feeder pigs caused by lightning strike. J Swine Health Prod 1993;1:36.

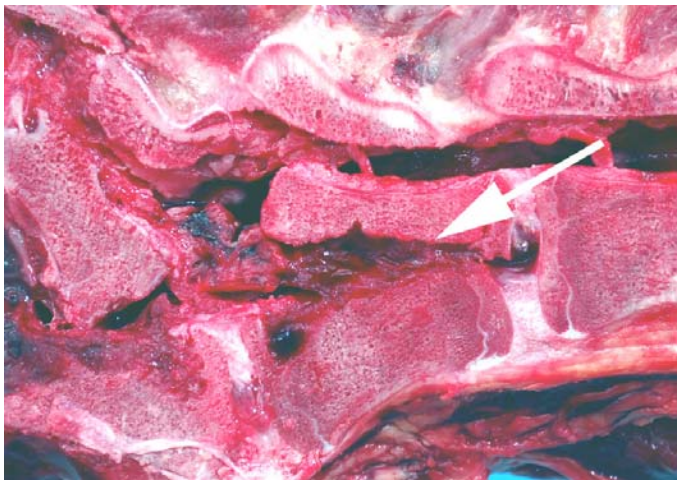


Figure 1. Longitudinal fracture (arrow) of the caudal lumbar vertebrae associated with lightning strike.

PVC-2 and PRRSV updates *Susy Carman, Beverly McEwen*

Figure 1. The percentage of **PCV-2 positive pathology cases** has declined from 10.1% in 2006 to 0.5% in 2011:

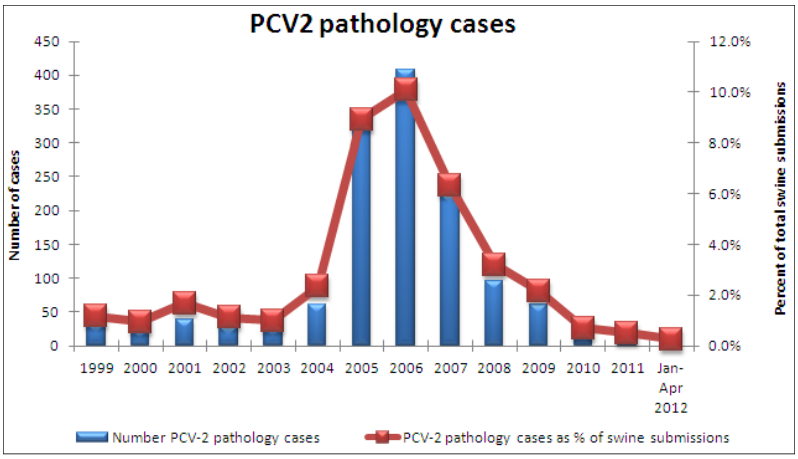
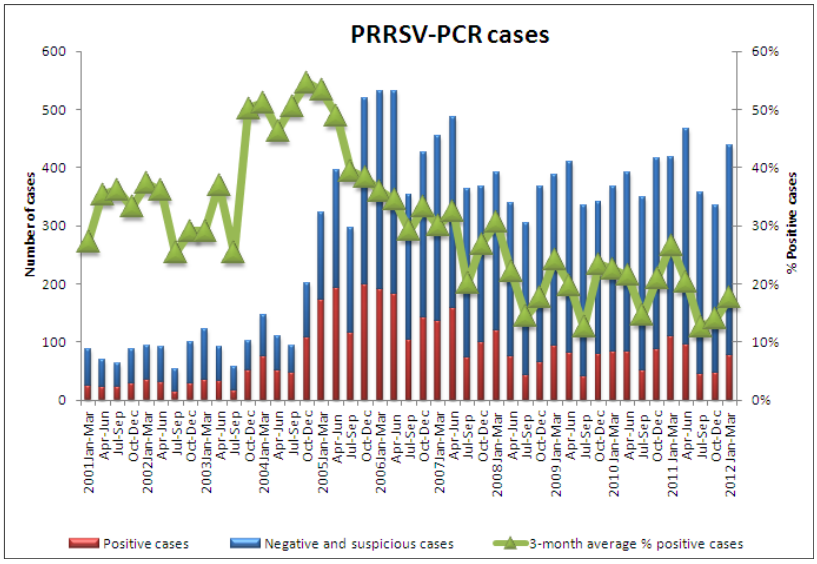


Figure 2. Average annual percentage of **PRRSV PCR-positive cases** continues to decline from a peak of 51% in 2004 to 18% in 2012:



HORSES

Equid herpesvirus-1 (EHV-1) surveillance update

Susy Carman, Beverly McEwen

Neuropathogenic EHV-1 was identified in samples collected on Jan 26, 2012 from 1 Ontario horse with neurological disease. This initiated an ongoing OMAFRA funded EHV-1 surveillance program, with all virus detection testing done at no charge for submitters. Since then, the AHL has tested samples from 9 additional horses with neurological disease, 2 horses in contact with a horse with neurological disease, 13 aborted equine fetuses, 14 adult horses with respiratory disease, 1 neonatal foal with respiratory disease, 1 dummy foal, and 5 horses with no history included on the submission form. **EHV-1 was identified using real-time PCR in 1 aborted fetus and in 3 horses with respiratory disease. All 4 strains were typed as non-neuropathogenic using PCR.** Since 2007, 4 neuropathogenic and 15 non-neuropathogenic strains of EHV-1 have been confirmed at the AHL using PCR.

Despite the OMAFRA-funded enhanced surveillance initiative, similar numbers of tests were conducted in Jan, Feb, Mar 2012, as for previous years. An average number of 16 PCR tests were requested for each month.

The percentage of AHL equine pathology cases found to be EHV-1 positive by FA, IHC, or PCR testing (Figure 1) over the last 5 years was 3% (2007), 7% (2008), 14% (2009), 4% (2010), 9% (2011). The percentage of tests positive in the first 3 months of 2012 was 7%. *AHL*

AVIAN/FUR/EXOTIC SPECIES

An outbreak of *West Nile virus* infection in farmed domestic ducks in Ontario

Margaret Stalker, Emily Martin, Marina Brash, Davor Ojkic, Alex Weisz

In October 2011, the AHL received formalin-fixed tissues collected from a flock of 600, 6-week old domestic ducks experiencing an increase in mortality. Affected ducks displayed general weakness and inability to stand (Figure 1a). Flock mortality over a 4-week period reached approximately 15%. Enlarged, flaccid hearts with biventricular dilation, myocardial pallor, and hydropericardium were noted on field necropsies (Figure 1b). Histologic examination of the hearts of 3 ducks revealed lesions ranging from patchy degeneration of cardiac myocytes with early mineralization, to extensive areas of myocardial atrophy and fibrosis with an infiltrate of predominantly mononuclear inflammatory cells. In addition, there was widespread lymphocytolysis within sections of spleen. Differential diagnoses considered included ionophore toxicosis or nutritional myopathy, as well as infectious myocarditis.

Immunohistochemistry (IHC) for *West Nile virus* revealed abundant positive staining for viral antigen within each of the sections of myocardium (Figure 2a), with additional staining of the vascular tunica media in parenchymal blood vessels of the spleen. Further follow-up necropsies revealed minimal nonsuppurative encephalitis in one section of brain, as well as myocardial and splenic lesions. Occasional apoptotic hepatocytes were present in sections of liver, and similar single dead cells were present in sections of pancreas, while 1 section of cecum examined had acute typhlitis with necrotic cells in crypts. WNV was confirmed by real-time PCR (rt-PCR) from pooled tissues from these birds, and viral antigen was detected by IHC in intestinal epithelium (Figure 2b), Kupffer cells of the liver, interstitial cells in the pancreas, and small glial foci in the brain. Additional rt-PCR tests for APMV-1 and *Influenza A virus* were negative.

This is the first report of clinical disease associated with WNV infection in domestic ducks in Ontario. Outbreaks of disease due to WNV infection in domestic poultry are seen primarily in domestic geese; the only other published reports of disease in ducks are from Saskatchewan, documenting WNV infection in a flock of domestic ducks and a flock of captive lesser scaup during the summer of 2007. *AHL*

References

Himsworth CG, et al. An outbreak of West Nile virus infection in captive lesser scaup (*Aythya affinis*) ducklings. Avian Dis 2009,53:129-134.

Wojnarowicz C, et al. First Canadian outbreak of West Nile Virus disease in farmed domestic ducks in Saskatchewan. Can Vet J 2007,48:1270-1271.

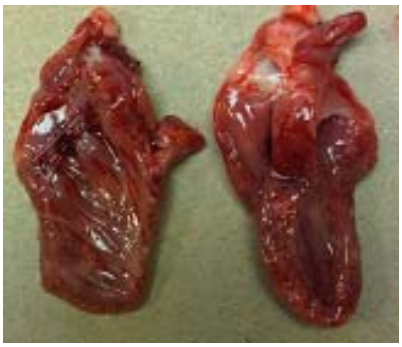
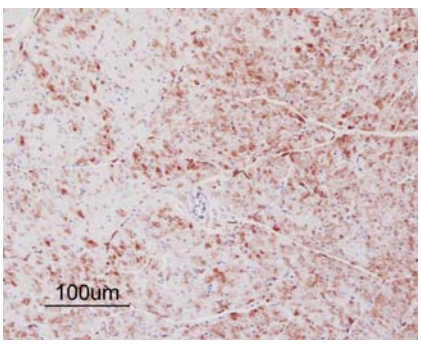
a  b 

Figure 1: (a) Clinically affected duck displaying profound weakness; (b) Enlarged, flaccid heart with multiple foci of myocardial pallor. (Images courtesy of Dr. A. Weisz).

a 

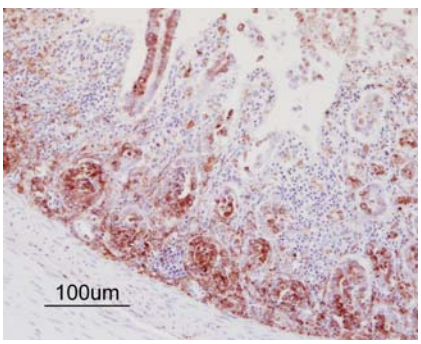
b 

Figure 2: Abundant staining for West Nile viral antigen present in the (a) myocardium and (b) intestinal epithelium of affected ducks.

COMPANION ANIMALS

Copper-associated liver disease in the dog: hepatic copper assessment

Brent Hoff, Nick Schrier, Margaret Stalker

Chronic liver disease associated with abnormal hepatic copper storage was first identified in Bedlington terriers. Affected dogs typically have an inherited autosomal recessive defect in the gene *COMMD1*, encoding a protein thought to be involved in hepatic copper transport and excretion into bile. Chronic hepatitis with hepatic copper accumulation is also recognized in other breeds, including the West Highland white terrier, Skye terrier, Doberman pinscher, Dalmatian, and Labrador retriever. However, the inheritance and functional alteration in hepatic copper metabolism in these breeds is incompletely understood, as copper accumulation may also occur as a secondary phenomenon in chronic liver disease due to cholestasis or canalicular damage.

Affected dogs may be presented with increased ALT and ALP activity, and in early stages this may be the only abnormal finding, however those seen later in the course of disease may demonstrate the full range of clinicopathologic features consistent with chronic hepatitis and cirrhosis. Histologic evaluation of liver tissue is required for a definitive clinical diagnosis of the disease.

Confirming a diagnosis of copper-associated liver disease

Liver biopsy is the only way to substantiate hepatic copper accumulation. Formalin-fixed liver biopsies are useful for routine histologic examination, allowing evaluation for evidence of liver injury, repair, inflammation and fibrosis, as well as qualitative estimates of copper accumulation and zonal distribution by the use of special stains (copper is detectable by rhodanine stain at concentrations >400 ug/g) (Figure 1). In addition to biopsy samples collected for histology, 1 or 2 additional liver biopsies (10 mg) taken with a Tru-Cut®or Surecut® disposable biopsy needle will yield adequate tissue for quantitative copper analysis. Using a hypodermic needle, remove the liver biopsy sample for copper analysis and transfer into a test tube. It is best not to use rubber-stoppered tubes as they have potential to leach trace elements and cause potential contamination. Test tubes that have plastic stoppers or nylon transfer tubes are preferred.

Copper assessment

Copper concentrations in liver can be assessed quantitatively by ICP/MS on a very small amount of liver tissue (5 mg dry weight). Formalin fixed tissue can be used, but some accuracy may be lost. The average liver copper concentration in normal dogs is 200 to 400 ug/g dry weight. For breed-specific reference values, see reference below.

Sending samples to us

The test tubes containing the liver biopsy sample should be sent to the laboratory using an overnight courier. Please **DO NOT** add formalin, saline or anything else to samples intended for copper analysis. Samples are to be slightly chilled over ice packs or frozen. Submission forms can be obtained on-line, or contact us at 519-824-4120 ext. 54530 and we will gladly fax or mail you customized submission forms. *AHL*

Reference

Hoffman G, et al. Copper–associated chronic hepatitis, Kirk's Current Veterinary Therapy XIV, St. Louis, Saunders/Elsevier, 2009.

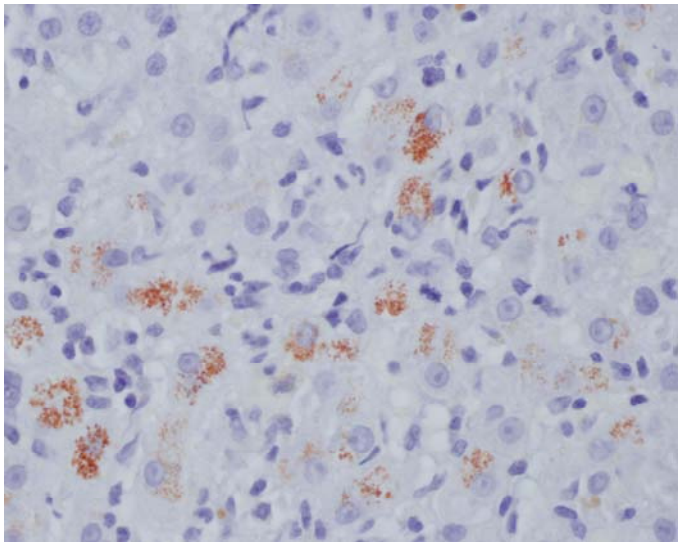


Figure 1. Copper accumulation in hepatocytes in a canine liver biopsy (60X magnification, rhodanine stain).

AHL Newsletters and LabNotes are available on the Web at - http://ahl.uoguelph.ca