JNIVERSITY

25

Laboratory Services Division

#### Animal Health Laboratory





# **AHL** Newsletter

Canada Post Publications number - 40064673

Volume 18, Number 4, page 25

December, 2014

ISSN 1481-7179

#### 

AAVLD/JVDI Awalu	20
Susy Carman award Renovations	25 25
Ontario Animal Health Network	26 27
Ruminants Coxiella placentitis Orf/BPSV PCR Field PM images	28 29 29
Aquaculture - fish tests	29
Swine ITL cardiomyopathy	30
Horses Actinobacillus cellulitis EEE in Ontario, 2014	31 32
Avian/fur/exotic Leukocytozoonosis duck Enterococcus hirae	33 34
Honey bees - new tests	34
Companion animals Inking biopsy margins Bacteria urine sediment	35 36



Pioneers in Virology Award! Dr. Susy Carman, recently retired from the AHL, was recognized for her many contributions to veterinary virology at the October 2014 annual meeting of the American Association of Veterinary Laboratory Diagnosticians meeting in Kansas City, MO.

### Winners of the 2013 JVDI best paper award!

We're pleased to report that an AHL group received the AAVLD award for the best paper published in the Journal of Veterinary Diagnostic Investigation for 2013.

Hazlett MJ, McDowall R, DeLay J, Stalker M, McEwen B, van Dreumel T, Spinato M, Binnington B, Slavic D, Carman S, Cai HY. A prospective study of sheep and goat abortion using real-time polymerase chain reaction and cut point estimation shows *Coxiella burnetii* and *Chlamydophila abortus* infection concurrently with other major pathogens. J Vet Diagn Invest. 2013;25:359-68.



Above (L to R): Murray Hazlett, Beverly McEwen, Margaret Stalker, Josepha DeLay, Tony van Dreumel, Maria Spinato, Durda Slavic, Hugh Cai. Missing, Rebeccah McDowall, Brian Binnington, Susy Carman.



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

**Renovations!** AHL-Guelph Specimen Reception will be undergoing renovation December-February to improve both our biosecurity and our service efficiency. We will do everything possible to minimize inconvenience to our clients. Writing sample IDs on your submission forms or sending Excel spreadsheets to <u>specroom@uoguelph.ca</u> will help us serve you faster.

#### Ontario Animal Health Network - cont'd from p. 27

**Fish:** The fish expert network has started as of October 2014. With few veterinarians in the field in Ontario, a **custom approach was necessary** for this network. The group will be meeting quarterly to discuss what issues and diseases are present in labs (AHL, University of Guelph, OVC) as well as with-in agencies like OMNR, OMAFRA, and of course what is being seen in the field, while respecting **strict confidentiality**. **The AHL is also testing aquaculture fish for a wide variety of diseases**.

**Bovine:** The bovine network is under development. Currently, OAHN is **funding some case investigations into diarrhea in calves in Ontario**, and OAHN funded the summer mastitis project.

**Equine:** The equine network is under development. **OAHN will be sponsoring and presenting about OAHN at the Ontario Association of Equine Practitioner's Annual General Meeting** in February 2015. The event is co-sponsored by OMAFRA. OAHN has also produced an equine podcast about diagnosing and treating equine colitis. Click here to listen: <u>OAHN Podcasts</u>

#### AHL Newsletter

December, 2014 - Volume 18, Number 4

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. All material is copyright 2014. Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.

Articles may be reprinted with the permission of the editor and with appropriate credit given to the AHL Newsletter.

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

**Poultry:** At this time of year, poultry vets are asked to provide feedback about incidence of disease as part of the annual producer updates. This year, the survey was completed on-line and dovetailed with the AHL lab data as a first step in developing the OAHN program in the poultry industry. **Private practitioner participation in this survey was excellent, with 100% of vets completing the survey!** Results, report and program were discussed at the November 27<sup>th</sup> OAPP meeting in Guelph. We welcome your thoughts and look forward to the meeting as we move forward together.

**Bees:** Network under development. Bee testing is underway with a wide variety of tests available. The AHL offers diagnostic services for detecting many honey bee pathogens: *Nosema*, tracheal mites, and 7 major viruses of honey bees.

**Companion animals:** A podcast about rabies testing in Ontario will be available soon. Stay tuned for further developments in the OAHN companion animal network.

**Fur-bearing and alternative species:** Under development

Wildlife: Under development

Contributors to this issue - from the Animal Health Laboratory: Melanie Barham, DVM Patricia Bell-Rogers, BSc, MSc Marina Brash, DVM, DVSc, Diplomate ACVP Hugh Cai, DVM, MSc, DVSc Josepha DeLay, DVM, DVSc, Diplomate ACVP Jason Eidt, BSc Murray Hazlett, DVM, DVSc, Diplomate ACVP Megan MacAlpine, BSc, AHT Anna Marom, BSc, MSc Beverly McEwen, DVM, PhD, Diplomate ACVP Davor Oikic. DVM PhD Kristiina Ruotsalo, DVM, DVSc, Diplomate ACVP Durda Slavic, DVM, PhD Andrew Vince, DVM, DVSc, Diplomate ACVP Jennifer Zechel, PhD **Other contributors:** Monika Janssen, BScH, DVM, Neebing, ON Allan MacLeod, DVM Caledon, ON Alison Moore, DVM, DVSc, DACVIM, DACVSMR, OMAFRA, Guelph, ON Tony van Dreumel, DVM, MSc, Diplomate ACVP, Guelph ON Alex Weisz, DVM, Guelph, ON Kathy Zurbrigg, MSc, OMAFRA, Elora, 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.

### Ontario Animal Health Network - up and running! Melanie Barham

What is OAHN? OAHN is a new way for Ontario commodity groups to tackle important disease issues in their sector, collaborate with other industries, and access valuable resources. Each sector will have an "Expert Network" comprising an AHL, OVC and OMAFRA species specialist and up to 3 private practitioners. The Expert Network will meet quarterly to discuss pertinent diseases and issues affecting the sector. Laboratory data will be discussed, together with the results of a quarterly veterinary survey. The networks' focus is on identifying trends and actionable items for the industry, and they will work together with producer groups. Networks will also participate in cross-species information sharing, and the OAHN plan will be able to link with other provinces and a national program as these initiatives are developed.

**How does OAHN impact me?** Each quarter, an email request will be sent to the veterinary listservs to ask practitioners to complete a survey about the issues and diseases you are seeing in your practice. The surveys take 5 minutes or less to complete and can be completed on a desktop computer, tablet,or smartphone. If you are part of a veterinary group like OASV, SRVO or OAPP, you may have already received some emails asking you to complete a survey, or received a veterinary report. Your response is crucial! After the survey has been completed, the Expert Network meets and a veterinary report is created and circulated to the appropriate veterinary listserv, summarizing issues across Ontario. Relevant papers, client resources, podcasts may accompany the reports, so that in addition to an excellent summary of current disease and industry issues in Ontario, the reports are a valuable tool to help you make decisions with your clients.

Top 10 reasons to be involved:

- 1. Strengthens veterinary relationship and role with producer groups and clients
- 2. Open, personal communication among industry, government and academic resources
- 3. Connection to funds and resources for follow-up of issues
- 4. Coordinated risk assessment automatically places veterinarians at the heart of the answer
- 5. Automatic connection to industry, provincial and federal resources
- 6. Access to technology and tools to communicate better and make better decisions
- 7. Cross-species sharing of information
- 8. Emergency preparedness
- 9. Effective leverage for funds that matter
- 10. Strengthens veterinary voice in Ontario

Questions? Comments? Would you like a copy of the reports? Do you want to be involved? **Contact Dr. Melanie Barham** at (519) 824-4120x53364 or <u>barhamm@uoguelph.ca</u>. Website: <u>OAHN Website</u> Podcasts: <u>OAHN Podcasts</u>



More expert networks!

**Small Ruminants:** The small ruminant expert network has been working together since August 2014, having met twice to discuss trends in sheep and goats from May-July and July- Sept. **Practitioners have been very involved and the enthusiasm of the team is "infectious."** Top issues included coccidiosis, hemonchosis, pneumonia and wasting. The full reports can be obtained by emailing the network coordinator. Small ruminant podcast can be accessed here: <u>OAHN Podcasts</u>. More podcasts are on the way! **Swine:** Survey and lab data have been compiled for July, Aug, Sept 2014 and the Expert Network met to discuss on November 5<sup>th</sup>. There was excellent discussion on the call, and a veterinary report will be released shortly. Further update on the program will be discussed at the presentation at the OASV meeting November 27<sup>th</sup>. OAHN data is being shared with CSHIN's national program. Ontario Pork and OSHAB are close advisors for this network and we look forward to a continued close relationship with producer groups. Ongoing surveillance testing for PED has been funded through OAHN.

... Continued on page 26.

## AHL Lab Reports RUMINANTS

### Perinatal mortality in a calf resulting from Coxiella burnetii placentitis

Andrew Vince

In September 2014, a full-term bull calf fetus and associated placenta were received by the AHL for evaluation after the loss of 4 other calves on farm over a 1- month period. Some calves were born alive but succumbed to progressive weakness, recumbency, and anorexia/lack of suckle reflex. Grossly, the placenta was thickened and covered with fibrin and foul-smelling purulent debris; the content of the abomasum was murky yellow and flocculent (usually clear and viscous), and the lungs were abnormally firm/rubbery and edematous. Bacterial placentitis was considered the principal diagnosis, and tissues were submitted for histopathology. Placenta, lung, and abomasal content were submitted for bacterial culture. Numerous additional tissues and fluids were frozen as per the AHL bovine abortion protocol (page 23 of the AHL User's Guide).

Histologically, **the fibrinopurulent placentitis was marked by numerous trophoblasts containing amphophilic cytoplasmic organisms typical of** *Coxiella burnetii* (Fig. 1). Modified acid-fast staining of placental smears found similar *Coxiella*-like organisms, and confirmatory testing of placenta via PCR found  $3.13 \times 10^7$  *C. burnetii* genome copies /µL within the placental tissue. Abortion secondary to *C. burnetii* placentitis was diagnosed.

*C. burnetii* is a zoonotic intracellular gram-negative bacterial pathogen that can infect mononuclear phagocytes and other cell types, most notable for 2 disease syndromes: placentitis-associated abortion in small ruminants, and Q fever in humans. In small ruminants, *C. burnetii* causes abortion storms, stillbirths, and weak-born lambs and kids, involving up to half of a flock.

Q fever in humans is a complex syndrome with varying manifestations (often varying by age), including pneumonia, hepatitis/cholecystitis, meningitis/encephalitis, myocarditis/pericarditis, chronic endocarditis or other vascular infections, and osteomyelitis/osteoarthritis. Infected pregnant women may also experience loss of pregnancy or fetal growth disturbances. The principal reservoir of infection is via inhalation of infectious aerosols or dust contaminated with fresh or dried birth fluids or feces from infected animals. The bacterium is extremely environmentally resistant, lasting months-years in spite of heat or desiccation. Concern over the use of this pathogen as a possible biological weapon has been raised, as the infective dose is reportedly very low.

Cattle are also recognized as a primary reservoir for *C. burnetii* by the American Centers for Disease Control and Prevention (CDC), but it is infrequently recognized as a cause of abortion in cattle. In a 2012 review of bovine abortion diagnoses at the AHL (AHL Newsletter vol. 16, no. 1), a single case of confirmed *C. burnetii* abortion was recognized out of 743 abortion submissions and 759 abortion diagnoses between 2006 and Jan 2012.

Because of the case described, AHL pathologists now perform postmortems on aborted calves within biological safety cabinets (in smaller submissions) or using positive pressure ventilation hoods (in larger submissions). **Primary care veterinarians are urged to use caution in similar situations**, in order to avoid infective exposure, and this **pathogen should be considered a differential diagnosis in clustered bovine abortions with placentitis**. *AHL* 



Figure 1. Bovine placenta, 200x magnification. The chorionic surface is covered by large quantities of fibrin and cell debris, and occasional intact trophoblasts contain blue-grey cytoplasmic organisms.

#### New PCR test for orf virus and bovine papular stomatitis virus

Murray Hazlett, Anna Marom, Davor Ojkic, Beverly McEwen

Submission of skin lesions for a confirmatory diagnosis of papular stomatitis or exudative exanthema (orf) due to parapox virus infection can be difficult unless typical inclusion bodies are seen in histologic sections. Usually, pathologists receive a section of very hyperplastic crusted skin, often with secondary pyoderma, lacking the pathognomonic inclusion bodies (Fig 1). When practitioners wanted to pursue an etiologic diagnosis, tissues were sent, usually to CFIA, for electron microscopy to confirm parapox infection and also rule-out other diseases such as foot and mouth disease depending on the clinical presentation.

We have recently developed a PCR test for parapox viruses that can be used on both formalin-fixed skin biopsy or crust material. The PCR test results were in 100% agreement with cases in which parapox infection was confirmed. In-province price is \$31 when testing fresh tissues and \$46 if done on a histology section. *AHL* 



Fig 1. Typical hyperplastic dermatitis with crusting and pyoderma without inclusion bodies in our most common presentation of an orf case. Because of the clinical presentation in the herd, this was sent to CFIA as a confirmatory negative for FMD. **Parapox virus** was demonstrated with EM.

#### Digital images enhance the value of field postmortems

#### Josepha DeLay

Dr. Eugene Janzen of the University of Calgary presented a practical approach to field post-mortem examination and imaging at the Ontario Association of Bovine Practitioners (OABP) fall continuing education meeting in Guelph on November 20, 2014. The AHL welcomes submission of digital images from field and clinic PMs from <u>all species</u> to accompanying histopathology submissions. Please send images to <u>ahlpath@uoguelph.ca</u> Also please note on the submission form that images have been submitted for the case.

AHL pathologists are testing a standardized protocol and image list for field and clinic PMs, and this information will follow in a separate email in the near future. The AHL also plans to develop an app for this purpose. Submission of good quality images from field PMs will increase the diagnostic value of these cases for practitioners, and the AHL pathology group is enthusiastic about this new endeavor. *AHL* 

## AQUACULTURE

#### Fish testing lab now open Hugh Cai, Patricia Bell-Rogers, Jason Eidt

With the assistance of Dr. John Lumsden and the Pathobiology Fish Lab, OVC, the AHL has set up a fish testing lab offering: fish postmortem examination, bacterial isolation and identification, histopathology.

Currently, the tests are offered only for fish raised for human consumption - **trout, char, and tilapia**. Submissions must be made through a veterinarian in order to provide a VCPR and have management advice available.

The AHL is also now offering fish **viral hemorrhagic septicemia virus (VHSV) PCR**. With the assistance of Dr. Kyle Garver (Fisheries and Oceans Canada) and Dr. John Lumsden, the AHL has validated and implemented a published VHSV real-time PCR assay that detects VHSV of all genotypes (Ia, Ib, II, III, IVa, IVb). The AHL VHSV PCR is SCC ISO/IEC 17025 accredited under the AHL flexible scope of accreditation for PCR assays. The price is \$36 per sample (or pool of samples); a \$20 sample processing fee applies to pooling of samples (maximum 5 fish per pool). *AHL* 

## SWINE

### Cardiac lesions associated with the in-transit losses and on-farm sudden deaths of market hogs

Tony van Dreumel, Kathy Zurbrigg

To investigate the cause of death of market hog transport losses, 130 postmortems (PM) were completed on hogs that died in-transit or were euthanized on arrival to one Ontario abattoir. Significant findings included: pulmonary congestion with or without edema (100%), multi-organ congestion (11%), limb fractures (11%), lung lesions (10%), *Streptococcus suis* cultured from lung (4%), splenic/gastric torsion or hepatic fracture (4%), and pleuritis (2%). The majority of in-transit loss (**ITL**) hogs had cardiac lesions consistent with a primary cause of death and it was determined that further examination of the hearts was necessary.

The hearts from 194 ITL hogs (120 of the ITL hogs had a routine PM performed) and 217 non-in-transit loss (non-ITL) hogs were examined using a standard protocol. Weights of the total heart (THW), left ventricle and septum (LV+S), and right ventricle (RV) were recorded. Comparison of a subset of hearts found that THW, THW/body weight ratio, and RV and LV+S weights were significantly greater for ITL hogs compared to non-ITL hogs (1). Gross cardiac lesions were identified in 191/194(98%) of hearts from ITL hogs. The characteristic lesions consisted of mild to marked enlargement of the heart (Fig. 1) and mild to marked thickening of the LV+S with or without dilation of the aorta, pulmonary artery, right atrium, and RV. Pericarditis (with mild to marked thickening of the LV+S) was recorded in 6/217 (3%) ITL hearts. Non-ITL hogs had 43/217 (20%) hearts with gross cardiac lesions. These lesions included: marked LV and/or RV thickening with or without mild RV dilation. Pericarditis (with mild to moderate thickening of the LV) was noted in 5 (2%) non-ITL hearts. Tissue sections were taken from the RV, anterior and posterior LV and septum for histopathology. Analyses comparing the prevalence and severity of histological lesions between ITL and non-ITL hearts have commenced.

The gross and histological lesions found in Ontario hogs are similar to those reported as hypertrophic cardiomyopathy (HCM) by Liu et al (2) while investigating the sudden death of hogs in a Taiwan research facility. The examination of hearts from a small number of near-market-weight hogs that died suddenly on several different Ontario farms has found cardiac lesions similar to those in hogs that died in -transit. HCM-like lesions should be considered as a possible cause of death in finisher pigs that die suddenly on farms or in-transit. These pigs may have pre-existing lesions which can result in acute heart failure. **Practitioners faced with sudden death in market hogs on the farm or in-transit should submit the entire carcass for PM examination.** If submitting the carcass is not practical or feasible, **the entire heart may be submitted in formalin**. When only the heart is submitted, please include an estimate of hog weight with the history. *AHL* 

#### References

1) Zurbrigg K, van Dreumel T, Friendship R, O'Sullivan T. Cause of death for hogs that die during transport to an abattoir. Proc Ann Mtg Am Assoc Swine Vet 2014:455-456.

2) Liu S, Chiu Y, Factor S, Chu R, Lin J, Hsou H, Fox P. Hypertrophic cardiomyopathy in pigs: Quantitative pathologic features in 55 cases. Cardiovasc Pathol 1994; 3:261-268.

Funding for this project was provided by an Ontario Pork Research Grant and by the Agricultural Adaptation Council's Canadian Agricultural Adaptation Program and Ontario Farm Innovation Program.



Figure 1. Enlarged ITL heart (left) compared with a non-ITL heart (right)

## HORSES

#### Fatal Actinobacillus cellulitis and laryngitis in a horse

Murray Hazlett, Allan MacLeod, Durda Slavic

A 14-year-old Thoroughbred gelding experienced a fever of 103 F (39.4 C) and swelling in the throat/right submandibular region. The horse was treated with phenylbutazone by the owner and the next morning the rectal temperature had returned to normal. The following afternoon the gelding was visited by the regular veterinarian. The gelding had generalized swelling around the medial side of the right hemi-mandible. The area was painful to touch. Ultrasound exam did not reveal any specific site of abscess formation. A respiratory exam was performed with findings within normal limits. An oral exam was performed with no abnormal findings. A nasal lavage sample was obtained for Streptococcus equi PCR, the horse was started on trimethoprim-sulfa and placed in a quarantine area on the farm. Four hours later the horse was visited again because of difficulty breathing. Severe inspiratory and expiratory stridor was present. Upper respiratory endoscopy displayed marked pharyngeal collapse. An emergency tracheotomy was performed but was unsuccessful. The horse was sent to the AHL for postmortem examination.

At autopsy, a 10 mL necrotic pocket was located medial to the right mandible, about 10 cm rostral to the caudal edge of the ramus, containing cloudy red fluid and with an irregular capsular surface and surrounded by edematous yellow connective tissue with some early fibroplasia. Surrounding, ventral and caudal to this there was extensive hemorrhage and edema in the intermandibular area that extended to the throatlatch and surrounded the larynx. Tissue in these areas was friable and hemorrhagic. The submandibular lymph nodes were edematous and red. Yellow edema fluid was present surrounding the larynx, including around the esophagus dorsal to the larynx and rostral trachea. The mucosa of the guttural pouches was congested and both this and the pharyngeal mucosa were covered with small amounts of mucoid brown fluid.

Histologic examination of laryngeal and perilaryngeal tissues revealed marked edema, congestion, and hemorrhage. There was **severe neutrophilic cellulitis**, and large numbers of neutrophils with macrophages were seen amongst the proteinaceous fluid and within submucosal lymphoid tissue. In the intermandibular pocket region there was severe edema and hemorrhage as well as fibroblast activation with fibrin effusion and neutrophilic inflammation. Large colonies of small gram-negative coccobacilli (Fig. 1) as well as some colonies of gram-positive cocci were seen. The abscess wall

had fibroblast activation and endothelial cell proliferation, but no significant collagen formation (suggesting an age of less than 5 or 6 days). There was very mild acute neutrophilic pneumonitis.

Bacteriologic culture recovered large numbers of Actinobacillus lignieresii-like organism and Actinobacillus equuli subsp. haemolyticus. In addition, mixed anaerobes were isolated. Although initially suspected clinically, no Streptococcus equi subsp. equi was identified on culture or via PCR testing of a premortem nasal swab.

*A. equuli* subsp. *haemolyticus* and *A. lignieresii*-like organisms have been associated with infectious disease in horses. *A. equuli* subsp. *haemolyticus* is associated with sporadic cases of endocarditis, meningitis, metritis, abortion, cellulitis, and respiratory and wound infections, whereas *A. lignieresii*-like organisms were isolated from abscesses and respiratory tracts infections. In general, cellulitis is not commonly associated with *Actinobacillus* spp. clinical disease. However, in our case, **severe clinical cellulitis occurred in a healthy adult horse with no known predisposing factors**. Given that *Actinobacillus* spp. are frequently isolated from the oral or upper respiratory tract of healthy horses, it is possible that there was a small puncture wound that led to localized infection with abscessation, and then more wide-spread cellulitis. *AHL* 



Figure 1. Severe cellulitis with degenerate neutrophils in submandibular region (H+E 20x). Insert shows typical colonies of gramnegative coccobacilli (B+H 60x).

### Eastern equine encephalitis in Ontario, 2014

Alison Moore

This year has the dubious honour of being the worst year for eastern equine encephalitis (EEE) in Ontario's history. Twenty-two horses and 2 emus in the province died or were euthanized due to the disease with potentially as many deaths being suspected by attending veterinarians. Two horses were confirmed infected but survived. Counties in Eastern Ontario suffered the greatest casualties. Diagnosis in 21 horses was by serum IgM ELISA testing and 3 were diagnosed by RT-PCR on brain tissue. The affected horses were diagnosed between the end of July and the end of October. Ages of affected horses ranged from 2-20+ years, with no breed or sex predilection. Most of the infected horses were unvaccinated backyard horses and only a single horse per property was clinically affected. Most horses had an acute onset of disease with death or euthanasia performed within 24-48 hours. Common clinical signs included ataxia progressing to recumbency, with fever noted in some and blindness and head pressing noted in others. In the 2 horses that survived, the clinical signs were mild (ataxia and lethargy). The 2 emus were diagnosed with hemorrhagic enteritis and EEEV confirmed in the intestine and liver by RT-PCR.

The virus causing EEE is transmitted by mosquitoes. In Ontario, the most important species is *Culiseta melanura*, which feeds on birds. Bridge vectors, mosquitoes that feed on both birds and mammals, then complete the cycle to humans and horses. Outbreaks occur in hardwood, flooded areas with competent avian reservoirs and mammals present. Horses and humans are dead-end hosts as they do not produce sufficient viremia to infect mosquitoes.

So why was 2014 such a devastating year? Some speculate that eastern Ontario was relatively warmer this year than other parts of the province, others say it was due to the amount of spring precipitation. Others implicate the spring migration of wading birds such as herons from Florida. Herons are a preferred host for *Culiseta* sp. over winter in Florida, a major reservoir state for EEEV. The spring migration of herons and similar birds is thought to disseminate the virus to the northern USA and Canada. OMAFRA and Public Health Ontario will be working together over the winter to determine any associations between ecological and meteorological factors and disease occurrence. Regardless of the risk factors, however, equine vaccination for EEE as per label directions is protective against this deadly disease and should be a part of a core vaccine strategy.

For more information, please see the OMAFRA site:

s Culiseta melanura, Equine Neurological Disease Surveillance 2014



#### How to print legal-sized EIAV ELISA lab reports Please see LabNote 31 on our Website - <u>ahl.uoguelph.ca</u> <u>http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote%2031%20EIA%20Printing%20on%20Legal%</u> <u>20Paper%20vs%20Letter%20Paperfinal.pdf</u>

## AVIAN/FUR/EXOTIC SPECIES

#### Leukocytozoonosis in a group of young Welsh Harlequin ducks from northern Ontario Marina Brash, Megan MacAlpine, Monika Janssen

A group of 11 Welsh Harlequin ducklings hatched in northern Ontario in late June were housed indoors until approximately 4-5 weeks of age, then allowed access to an outdoor enclosure during the day. Aside from green watery diarrhea, the ducks were clinically normal. However in mid-August, at 6 weeks of age, 1 of the ducks became inappetent and lethargic, exhibited laboured breathing, and died overnight. In the following 2 days, 3 more ducks died, the last 2 exhibiting similar clinical signs. **Five of the 11 ducks died**; 2 recovered following intensive supportive care. Chickens housed in a separate enclosure suffered no morbidity or mortality. Pens were cleaned every 1-2 days; feed and water were always available and refreshed daily.

Two ducks were received for postmortem examination at the AHL. Both ducks had similar findings and were in good body condition with good muscle mass, external and internal fat stores, but the tissues were generally pale. There was mild generalized subcutaneous edema and focal hemorrhage in the caudal pectoral muscle. The lungs were pink/ purple and markedly edematous. There was focal hemorrhage on the epicardium. Spleens were very enlarged and soft (Fig. 1). Livers were enlarged, tan/red and soft. Esophagi and proventriculi were empty; the gizzards contained grit and intestines contained only a small amount of bile. Kidneys and bone marrows were pale.

No bacterial pathogens were isolated on culture. PCR testing was negative for influenza A virus, avian paramyxo-virus-1, West Nile virus, and Eastern equine encephalitis virus.

Histologically, there was widespread multifocal to coalescent acute hepatic necrosis with mild to marked hemorrhage and hepatocellular dissociation. Sinusoids and vessels contained numerous red blood cells with eccentric compressed nuclei and round structures compatible with protozoal gametocytes (Fig. 2). Kupffer cells were enlarged and contained pale orange pigment, cellular debris and occasionally phagocytized erythrocytes. Numerous protozoal megaloschizonts were present in the brain, occasionally within capillary lumens, infrequently surrounded by a small amount of hemorrhage with a few mononuclear cells and granulocytes in the adjacent parenchyma (Fig. 3). Parasitized RBCs were in circulation in many tissues.

The megaloschizonts in the brain confirm that the intraerythrocytic gametocytes were *Leukocytozoon* spp. *Leukocytozoon simondii* is the species that infects ducks and geese. The principal clinical effect of *Leukocytozoon* spp. infection is intravascular hemolytic anemia related to the release of an antierythrocyte factor produced either by the meronts or their host cells rather than by primary mechanical

destruction of the RBCs by the protozoa.

The vector for *L. simondi* is the **black fly** (*Simuliidae* spp) and infective sporozoites are carried in the salivary gland. In discussions with the referring veterinarian, the black fly population in the area appeared to be very low in early August, but in susceptible ducks, **very limited exposure to infected blackflies is sufficient for the introduction of enough sporozoites to cause mortality**. The prepatent period for *L. simondi* infection is approximately 2 weeks, which fits well with the time from the release of the ducks into the outside enclosure and the onset of mortality.

The producer has been advised that leukocytozoonosis will need to be managed if she wishes to continue to raise susceptible ducks in northern Ontario. *AHL* 



Figure 1: Pleural spaces contained marked edema (\*); lungs were also very edematous and pink/purple (#). There was focal hemorrhage on the epicardium (white arrow). The spleen was very enlarged and soft (S). The liver was enlarged and tan/red (L).

Figure 2: Thyroid gland of duckling with intravascular parasitized RBC containing a round *Leukocytozoon simondi* gametocyte (yellow arrow) and eccentric compressed nucleus. Normal RBC (green arrow). 1000X H & E





Figure 3: Brain of duckling containing a *Leukocytozoon simondi* megaloschizont measuring 150 µm in diameter (black arrow). 400X H&E

### Enterococcus hirae-associated septicemia in 34 day-old broilers

Marina Brash, Durda Slavic, Beverly McEwen, Alex Weisz

Postmortem examination of routine mortality of 34 day-old broilers identified hepatitis and splenomegaly with subcapsular hemorrhage. Sections of liver and spleen were collected for histology and a swab of liver/spleen was taken for bacterial culture. Histologically, in multiple sections of liver, there was acute multifocal fibrinoheterophilic hepatic necrosis, increased numbers of intravascular heterophils and occasional small colonies of coccobacilli, and remaining liver sections contained numerous intravascular colonies of coccobacilli. All the splenic sections contained colonies of cocci or coccobacilli. A Brown and Hopps Gram stain confirmed the presence of variable numbers of gram-positive cocci in all the tissues (Fig. 1). Moderate numbers of *Enterococcus hirae* and low numbers of *E. coli* were isolated from the internal organ swab.

In the late 1980s, re-examination of strains of *Enterococcus faecium* resulted in the **identification of a new enterococcus species**, *Enterococcus hirae*. *E. hirae* is considered to be a normal inhabitant of the small intestine of 3-4-weekold broilers but has also been associated with growth depression, encephalomalacia, valvular/mural endocarditis, septicemia, and osteomyelitis in broiler chickens of various ages.

A review of commercial poultry submissions to the AHL revealed that *E. hirae* was isolated from 7 additional broiler submissions from January 2013 until late October 2014. Six were from flocks of broiler chickens  $\leq 14$  days of age with histories of elevated mortality, small chicks and lameness. In these cases, *E. hirae* was isolated in variable numbers either in pure culture or in mixed culture with variable numbers of *E. coli* from heart, heart blood, bone marrow, blood-filtering organs, and joints. One case was from a 20-day-old flock of broilers with a history of ongoing leg issues and septicemia with yolk sac involvement; large numbers of *E. coli* and *E. hirae* were isolated from heart blood and bone marrow.

*E. coli*-associated septicemia is the most common type of bacterial infection seen in Ontario's commercial broilers and treatment typically includes the administration of a broad-spectrum antimicrobial such as trimethoprimsulfa. *Enterococcus hirae* is a gram-positive coccus that is considered inherently resistant to trimethoprim-sulfa, lincosamides including lincomycin, aminoglycosides including neomycin, kanamycin, gentamicin and tobramycin, and all cephalosporins. With these mixed bacterial septicemias, one can hypothesize that **response to standard treatments for** *E. coli* septicemia may be less successful when *E. hirae* is present, and that it may depend on the relative proportion of *E. hirae* to the overall intravascular bacterial load. *AHL* 



Figure 1: Spleen: Numerous colonies of intravascular gram positive cocci. 1000X Brown and Hopps.

### HONEY BEES



Animal Health Laboratory University of Guelph Tel: 519-824-4120 ext. 54530 Email: ahlinfo@uoquelph.ca

#### Honey bee testing Hugh Cai, Jennifer Zechel

The AHL now offers diagnostic services for honey bee testing including:

- quantitative PCR for honey bee viruses (acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Israeli acute paralysis virus, Kashmir bee virus, sacbrood virus)
- \* PCR for *Nosema* (*N. apis* and *N. cerenae*)
- \* microscopic examination for tracheal mites and Varroa mites
- \* quantitative testing of vitellogenin mRNA for honey bee health monitoring.

We currently offer these testing services on a project basis. For information on submission and pricing, please contact the AHL Molecular Biology Lab ahlmolec@uoguelph.ca AHL

## **COMPANION ANIMALS**

Surgical biopsies: Inking for margin evaluation Josepha DeLay

Surgical margin inking of biopsy samples allows the pathologist to definitively identify the margins in histologic sections and to evaluate completeness of surgical excision. At the AHL, margins of all excisional surgical biopsies are inked prior to trimming and processing of the samples. Inking at the time of surgery, however, is superior and provides more accurate results, as formalin fixation changes the size, texture, and color of biopsy specimens and can make accurate margin identification difficult post-fixation. Inking at surgery is especially helpful for small biopsies for which orientation is difficult after formalin fixation, such as equine third evelid biopsies and other biopsies from mucosal surfaces or non-haired skin. Marking the margins with ink aids in communication between the surgeon and pathologist regarding specific surgical margins of concern, and can provide biopsy orientation. Use of more than one ink color allows specific labelling of various margins for orientation and flagging purposes. For example, a margin suspected of being close or incomplete at surgery can be inked with blue, while the remaining margins can be inked with another color.

Surgical 'tissue dyes' are available from several suppliers, and waterproof artists' inks ('India ink') have also been successfully used for this purpose. Blue, green, and black are the most useful colors for identifying surgical margins in routinely stained biopsy specimens - best to not use black in melanotic tumors.

Biopsy inking is a simple procedure. Immediately after excision, the biopsy margins are first blotted (not rubbed) dry to remove blood and other surface debris. A cotton swab dipped in a generous amount of ink is gently rolled over the surgical margins, coating the entire marginal surface of the biopsy (Fig. 1). The ink must be allowed to dry for 5-7 minutes prior to placing the biopsy in formalin, in order to ensure that the ink coating remains intact during fixation. There will be some discoloration of formalin by the ink, but this will not impair fixation. When incising large biopsies to allow for better fixation, incision at the epidermal or superficial (rather than deep) aspect of the biopsy is recommended, to avoid ink penetration along nonsurgical incisions. When more than 1 color of ink is used to denote different areas or features of the biopsy, these colors and their significance must be recorded and included with the clinical history provided to the pathologist. Microscopically, the ink label allows confirmation of surgical margin location and dimensions (Fig. 2). AHL



Figure 1. Application of marking ink (tissue dye) to the lateral and deep margins of a biopsy following surgical excision.



Figure 2. Microscopic identification of a margin labelled with blue ink (blue arrow) allows evaluation of the surgical margin width surrounding an excised lesion (black arrow; in this case, a benign follicular cyst).

35

### Significance of bacteria in urine sediment

Kristiina Ruotsalo, Durda Slavic

In veterinary medicine, evaluation of urine sediment is frequently performed in the clinic to help determine if urinary tract infection is present. In order to assess the significance of bacteria in a urine sample, both the method of sample collection, and the concurrent presence of inflammatory cells must be considered. Urine is normally sterile until it reaches the mid-urethra. The urethra of dogs and cats contains a resident population of bacteria, with the greatest numbers at the distal end. Urine may also be contaminated by bacteria post-collection. **Thus bacteriuria in the absence of inflammation must be interpreted with caution**.

Rarely, dogs and cats may have bacteriuria without accompanying inflammation; these patients may be immunosuppressed or receiving medications such as glucocorticoids.

The lack of visible bacteria in the presence of inflammation also does not rule out septic inflammation. Given the detection limits of optical microscopy, there needs to be, on average, 10<sup>5</sup> CFU/mL of urine in order to identify these organisms. Additionally, detection of bacteria highly depends on the experience of the person interpreting the urine sediment preparation. Very frequently, different artifacts such as stain sediment can be misidentified as bacteria.

Finally, not all inflammatory processes may be accompanied by bacteria; for example urinary tract neoplasia or urolithiasis may occur with increases in leukocytes, erythrocytes and protein but not bacteria.

**Meaningful interpretation of a urine sediment preparation requires standardization of the procedure**; centrifugation of a standard volume of urine at a standard G force, removal of a consistent volume of supernatant, addition of a consistent amount of stain (if desired), transfer of the same number of drops of stained sediment, and use of a standard coverslip (22 X 22 mm). The importance of standardization of technique cannot be over-emphasized.

The sediment slide should be examined first at low power (4X or 10X objectives), using low light and a slightly lowered condenser, looking for casts, epithelial cells, and then at 40X objective for leukocytes, erythrocytes, and bacteria. Bacteria will be smaller than the erythrocytes, and always appear dark blue when Sedi-stain is used (this is NOT an indication of Gram-stain status, which requires a completely different staining technique). Bacteria will be round (cocci; Fig. 1A) or rice-shaped (rods; Fig. 1B). The finding of intracellular bacteria within leukocytes supports in vivo phagocytosis rather than contamination of the sample during collection or analysis.

The addition of a urine sediment stain (e.g., Sedi-Stain) is a matter of personal preference. **Such stains often contain abundant granular debris.** Thus allowing this material to settle to the bottom of the container, and gently pipetting stain from the top of the container may minimize the transfer of contaminants. As well, periodically checking the stain itself by applying a few drops onto a slide, coverslipping, and viewing under the microscope is important to ensure that no contaminants (bacteria or yeast) are present in the stain.

In summary, the detection of bacteria in fresh urine sediment should prompt consideration of a urinary tract infection, but **bacteria need to be interpreted in the context of sample collection and clinical picture**. Urine culture should be performed to confirm this finding. *AHL* 



Fig. 1. Modified Wright's stain of urine sediment from two clinical cases submitted to the AHL with A) cocci and B) rods observed in smears and subsequently confirmed by bacterial culture.

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