Laboratory Services Division

UNIVERSITY & GUELPH

Animal Health Laboratory



AHL Newsletter

Canada Post Publications number - 40064673



External audits Elizabeth King

AHL-Guelph and our Agriculture and Food Lab (AFL) sites had our biennial Standards Council of Canada (SCC) and Canadian Association for Laboratory Accreditation Inc. (CALA) audits the first week of October. Both audits were a success, and we received commendations from both the SCC and CALA audit teams. We are audited every 2 years by SCC and CALA as our quality system and our testing are accredited to the International Standard ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories* as shown on our scopes of accreditation (See our AHL website at https://www.uoguelph.ca/ahl/about-us/accreditation).

The audit teams include technical assessors who are experts in the various fields of testing being audited to ensure that technical requirements of ISO/IEC 17025 are also met. Each day for 5 days we had 2 to 6 auditors looking at processes and records for each lab area, specimen reception, client services, facility, human resources, information technology (IT), purchasing, quality assurance (QA) - including our internal audit program, as well as verifying management commitment to our quality system. Before SCC and CALA return in the fall of 2019, our lab will also be audited by the American Association of Veterinary Laboratory Diagnosticians (**AAVLD**) to maintain our "Full Accreditation / All species" for animal health testing. These external audits, like our client feedback, keep us moving forward on our commitment to continual improvement. *AHL*

Cold weather shipping reminder Jim Fairles

It's that time of year again when we need to start thinking about preventing samples from freezing. Specimens such as EDTA blood are rendered useless when frozen. Formalin will also freeze, which creates artifacts in fixed tissue. It can be difficult to protect samples shipped during the winter from severe cold. Even 10% neutral-buffered formalin will freeze in harsh winter weather conditions. To inhibit or reduce formalin freezing, add 1 mL of ethanol per 10 mL of formalin. Samples that should not be frozen should be shipped inside insulated containers with minimal cold packs. *Use of room temperature cold packs will help prevent temperatures from dipping too low.* If you have any concerns about the best way to ship critical samples, please contact the AHL. <u>ahlinfo@uoguelph.ca</u>

More submissions needed for the Adult Small Ruminant Mortality

Project! Maria Spinato, Jocelyn Jansen, Paula Menzies, Andria Jones-Bitton, Jeanette Cooper

The AHL, OVC, and OMAFRA have received funding to conduct a joint study investigating adult small ruminant mortalities. The objectives of this study are:

- 1. To determine why adult sheep and goats are dying on-farm.
- To determine if a web-based postmortem information and case submission system can be used to increase the usefulness of on-farm postmortems.
- 3. To determine if better disease diagnoses can increase discussions between producers and their vets, so that they can create sound flock/herd health and biosecurity plans.

The project will fund:

- \Rightarrow tissue collection kit and shipping fee,
- \Rightarrow AHL laboratory test fees,
- \Rightarrow compensation for veterinarian performing the on-farm postmortem.

In return, the veterinarian will submit:

- \Rightarrow a complete set of fresh and formalin-fixed tissues, as per project protocol,
- \Rightarrow a set of 6 postmortem photographs that are uploaded to the website,
- \Rightarrow postmortem submission form with all required information, also uploaded to the website.

Funding support is being provided by the OMAFRA-University of Guelph Partnership KTT Program and the Ontario Animal Health Network. Thanks to the generosity of these agencies, ~170 postmortems of adult sheep and goats will be paid for by project funds. A standardized test panel has been developed to check for the diseases of greatest importance to the small ruminant industry.

The maximum number of case submissions per veterinarian has been increased from 3 to 6 cases (must be a different clinical problem if multiple submissions per farm).

For more information, contact the project administrators at <u>sr.mort@uoguelph.ca</u> or log onto the project website at <u>https://www.uoguelph.ca/srmort/veterinarian/</u>

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Distance Support for On-Farm Investigation of Adult Small Ruminant Mortalities



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



This fall, we continued to spread the word about diseases that are concerning producers, pet owners, and veterinarians in Ontario and neighboring provinces. We have listed the diseases, links, and some details about each alert below. Many of our networks published reports this past quarter, with new reports from our Companion Animals, Equine, Poultry, Small Ruminants, and Swine networks. You can access producer and owner reports through our website at <u>OAHN.ca</u>, and if you are a veterinarian in North America, or an RVT in Ontario, you can view clinical impression summaries, lab data summaries, and veterinary reports by signing up and logging in. In January, OAHN will be hosting its annual workshop, bringing together producer groups, veterinarians, researchers, and members of government to plan for the coming year. If you want to keep on top of what we're up to, make sure you check out the News section on our site, for animal disease updates, report releases, and our weekly top animal health links of the week article.



Focus on equine strangles

We have published a new podcast series on equine strangles. In this series, our Equine Network answers horseowners' questions about equine strangles. We have private

practitioners, OVC researchers, and an OMAFRA vet answering everything from what strangles is to biosecurity practices and vaccines.

Listen to the full <u>5-part series here</u>.

To check out all of our podcasts, go to

OAHN.podbean.com. In addition to our most recent strangles series, we also have a 4-part series on Raw Food diets, covered from a nutritionist and an infectious disease perspective, information on honey bee resources in Ontario, and much more!

Benefits of registering on OAHN.ca

If you're a veterinarian in North America, or if you're an RVT in Ontario, you are eligible to register for <u>OAHN.ca</u>.

Registering for OAHN.ca gives you access to:

- \Rightarrow Veterinary reports and resources
- ⇒ Clinical impressions summaries from quarterly veterinary surveys
- \Rightarrow Quarterly lab data summaries
- \Rightarrow Condemnation data
- \Rightarrow Veterinary courses (such as our small flock veterinary course)
- ⇒ Disease updates meant only for veterinary professionals (e.g., CSHIN PED Outbreak Updates)

Disease alerts and industry updates

OAHN regularly publishes disease alerts and industry updates that are sent to us from OMAFRA, the Feather Board Command Centre, Public Health Units, and other reputable Ontario organizations. In the past quarter, we have published articles that affect many species groups:

- <u>Cluster of Pediatric Blastomycosis Cases</u>: A cluster of 3 confirmed cases and 1 suspect case of blastomycosis were found in pediatric residents of the Manitoulin District.
- <u>Atypical Porcine Pestivirus</u>: Atypical porcine pestivirus (APPV) was reported in a case of congenital tremors in piglets from gilt litters at a farm in Quebec. This is the first such report in Canada but has been identified in several US states, Europe, and China.
- <u>Avian Influenza Reminder for Poultry Farmers</u>: Information on how AI is spread, and what poultry farmers can do to reduce the risk of infection.
- <u>Confirmed Case of Epizootic Hemorrhagic Disease in</u> <u>Wild White-tailed Deer in Southern Ontario</u>: The Canadian Wildlife Health Cooperative reported the first occurrence of epizootic hemorrhagic disease (EHD) in 2 wild white-tailed deer in London, ON.
- <u>OMAFRA Equine Health Advisory: Eastern Equine</u> <u>Encephalitis Confirmed in Bruce County</u>: Information about the case found in Bruce County, vaccines available, what vets and horseowners can do, and a history of EEE in Ontario.
- <u>Industry Infectious Laryngotracheitis (ILT) Disease</u> <u>Advisories</u>: The FBCC has issued ILT advisories for Waterloo County, Lanark County, and the United Counties of Prescott and Russell.

New Blog Posts

Each month we will be posting new blog-style articles on OAHN.ca. Most recently, we published an article about CEZD, entitled: Community for Emerging and Zoonotic Disease (CEZD) – What It Is and How to Get Involved.

Do you ask your clients for their Premises IDs? Check out how fast and easy it is to do by registering your vet clinic: <u>https://www.ontarioppr.com/home_en.html</u>

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AHL Lab Reports RUMINANTS

Attention small ruminant practitioners! Suspected Cache Valley virus abortions in southern Ontario *Emily Brouwer, Courtney Schott*

Since the outbreak in 2015-2016, small ruminant producers, practitioners, and pathologists have been vigilant in monitoring small ruminant abortions for fetal deformities associated with Cache Valley virus (CVV). An update published in the March 2017 issue of this newsletter identified 2 ovine fetuses with craniofacial and spinal deformities that were submitted for examination between December 2016 and February 2017. Both of these lambs subsequently tested negative for CVV via serology.

Recently, a set of quadruplet lambs were submitted for postmortem examination. The history provided indicated that the lambs had multiple skeletal deformities, and were either born dead or immediately euthanized at lambing. The external lesions in these lambs varied in severity and included combinations of pronounced lordosis/kyphosis (2 lambs), sternal malformation (2 lambs), subjectively long

limbs (4 lambs), and arthrogryposis (3 lambs). Internally, lesions were limited to the brain, calvaria, and skeletal muscle. Brain lesions included hydranencephaly, microencephaly, cerebellar hypoplasia, mild lissencephaly, and suspected holoprosencephaly (Fig. 1). The skeletal muscle in all lambs was markedly pale, firm, and shrunken.

The top differential diagnosis is in utero neurotropic virus infection, of which Cache Valley Virus is the most likely etiology. Serologic tests for fetal antibody to CVV and PCR for viral antigen are pending. **This update is to serve as a reminder to practitioners and producers to consider CVV in cases of ovine abortion where there are skeletal (Fig. 2) and neurologic deformities.** Lambs are commonly born at term and may be alive, but they may also be aborted and lesions vary in severity between lambs. *AHL*



Figure 1. Brains from quadruplet lambs. Brain lesions included hydranencephaly, microencephaly, cerebellar hypoplasia, and mild lissencephaly





Figure 2. Aborted lamb with kyphosis and arthrogryposis. Photo courtesy of Dr. M. Spinato.

December, 2017

AVIAN/FUR/EXOTIC SPECIES

Blackhead (histomoniasis) in small turkey flocks

Marina Brash, Lloyd Weber

Blackhead disease, also known as histomoniasis and enterohepatitis, is a disease that is diagnosed frequently at the AHL in late summer and fall in small turkey flocks, and because there are no approved preventive or treatment medications, mortalities can persist resulting in very high losses.

Blackhead is caused by a protozoan parasite, *Histomonas meleagridis*, which is carried by the common poultry cecal worm *Heterakis gallinarum*, found in the ceca of chickens and turkeys. *Histomonas* is fragile and cannot live outside the bird host for long but can survive for long periods of time in the environment when in the cecal worm or its eggs. Earthworms can also carry infected cecal worm eggs and are important in transmitting blackhead. Flies, beetles, including darkling beetles, grasshoppers, sowbugs, and crickets can also serve as mechanical vectors. Cecal worms and eggs can also be transferred via contaminated manure on equipment and shoes.

Typically, **outbreaks in turkeys begin with the ingestion of infected earthworms or cecal worms or eggs from contaminated soil or concrete-floored pens where flocks of chickens or turkeys have been raised.** Wild grouse and quail may also carry the infection to turkey yards. In turkeys, bird-to-bird transmission, through direct cloacal contact or with infected droppings, can contribute to maintenance of the outbreak. Chickens, pheasant, partridge, and peafowl are also susceptible.

Once the *H. meleagridis* organisms are ingested, they travel to the cecum, penetrate the mucosa, multiply, resulting in necrosis, inflammation, and thickening of the cecal wall, enter the bloodstream and travel to the liver inciting necrosis and inflammation. In the intestine, interaction with coccidia and bacteria including *Clostridium perfringens* and *E. coli* increases the severity of disease with more tissue damage. Turkeys can show clinical signs of illness 7-12 d post-ingestion including anorexia, drowsiness, ruffled feathers, drooping of the wings, placement of the head down and close to the body or tucked under the wing, and the excretion of yellow feces described as sulfur-yellow droppings (Fig. 1). The head may or may not be cyanotic. Death follows shortly thereafter.

At postmortem, lesions are characteristic with **enlargement of the cecal pouches and marked thickening of cecal walls with thick caseous cores** (Fig. 2). Extension of inflammation through the cecal serosa will result in localized peritonitis. The liver is enlarged, with multiple circular depressed areas of necrosis circumscribed by raised yellow rings described as **targetoid lesions** (Fig. 1). Lesions can also be found in other organs, including kidneys, bursa of Fabricius, spleen, lungs, pancreas, and proventriculus. Histo-

pathology can confirm the diagnosis as the histomonad organisms will be present in cecal and liver tissues.

There are no approved preventive or treatment medications, therefore prevention of this disease is the key and the approach is multipronged.

(For more detail on recommendations, please see our LabNote 54.)

- 1. Do not raise turkeys where chickens have been housed.
- 2. Concrete-floored pens are preferred to dirt floors.
- 3. Prevent fecal contamination of bedding and housing.
- 4. The cecal worm burden can be evaluated by inspection of cecal contents during PMs. Ascarid and cecal worm eggs can be differentiated by size on fecal flotations.
- 5. Moving sick turkeys will only contaminate the new site.
- 6. Contaminated litter must be removed. Washing and disinfecting concrete or wood floors works well.
- 7. If possible, move up the processing date for the remaining healthy birds. If the turkeys are dehydrated and/or in poor body condition at processing, and/or have grossly affected internal organs, they will be condemned. *AHL*



Figure 1. Sulfur-yellow droppings on the underside of the tail feathers. Typical targetoid lesions in liver.



Figure 2. Enlarged cecal pouches containing typical caseous cecal cores.

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SWINE

Ionophore toxicity in pigs

Josepha DeLay, Maria Spinato

Over a 6-day period, bodies or field postmortem samples of nursery pigs from 4 herds were submitted to the AHL. The clinical complaints ranged from weak and fading pigs, to animals that were stumbling or recumbent and demonstrated unspecified neurologic signs. Up to 30% morbidity and 20% mortality was reported in some herds. Antibiotic therapy was unsuccessful in resolving clinical signs in those herds that carried out this treatment.

Discussion among several of the swine veterinarians involved in these cases identified a common feed source, and further investigation determined that an ionophore had been added inadvertently to feed containing tiamulin. At postmortem, no significant gross lesions were evident in the pigs, however all animals had microscopic lesions involving striated muscle, with sparing of myocardium. Acute-to-subacute myodegeneration varied in severity among individual animals, and targeted skeletal muscle, as well as diaphragm and tongue in some animals. In addition to ionophore toxicosis, nutritional myopathy caused by selenium and/or vitamin E deficiency is a differential diagnosis for this histologic lesion; however selenium levels were assessed to be normal by liver mineral analysis.

Tiamulin potentiation of the toxic effects of ionophores in swine is well recognized and has been documented previously. Tiamulin is thought to inhibit normal cytochrome P450 activity and ionophore metabolism. Confirmation of ionophore toxicosis requires analysis of the source feed or Van Vleet JF, Ferrans VJ. Ultrastructural alterations in skeletal stomach content (in cases where the suspect feed is still being fed), and tissues cannot be tested for ionophores. Species differences exist for susceptibility to ionophore toxi-

cosis, and swine are moderately sensitive to this toxin. Comparatively, horses are highly sensitive to ionophore toxicosis, and chickens are relatively insensitive.

Muscle lesions were minimal in follow-up muscle samples taken 4 weeks following ionophore exposure from pigs in a 5th affected herd, indicating that recovery may be possible in some animals if the source of the offending ionophore is removed before muscle injury is overwhelming.

These cases demonstrate the value of communication among practitioners, and between practitioners and diagnosticians, in solving disease problems. This event also highlights the importance of including muscle from several sites among formalin-fixed histology samples from field postmortems. In pigs, myopathy can easily be confused clinically with neurologic disease, and a diagnosis of myopathy will be missed if muscle samples are not included for histologic evaluation. AHL

References

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- muscle of pigs with acute monensin mycotoxicosis. Am J Pathol 1984;14:461-471.



Figure 1. Myodegeneration in skeletal muscle of affected pig. Numerous swollen myocytes with sarcolemmal contraction bands and vacuolation (arrows).



Figure 2. Normal skeletal muscle in age-matched nursery pig. Intact, happy myocytes.

HORSES

A case of eastern equine encephalitis Murray Hazlett, Jaimee Gardner, Davor Ojkic

A mature mare located southeast of Owen Sound and recently vaccinated for rabies was found in the field in lateral recumbency, unable to rise, and paddling. The day prior, the owner reported the horse was normal and did some mild lunge work. The horse would rise to sternal recumbency, but only briefly before falling to the right again. She was bright and alert, but would grind her teeth and was unable to drink. She was unresponsive after 6 h to IV flunixin and IM dexamethasone therapy and was euthanized. The brain was removed the next day and sent to the AHL for testing.

The day following euthanasia of this animal, another horse on the property was found with almost identical neurologic signs - down in lateral/sternal recumbency. The second horse was more mentally stuporous, unable to swallow, and was euthanized after 24 h of no response to therapy. This horse was disposed of without testing.

In the tested mare, histologic examination of the brain revealed severe, neutrophil-rich encephalitis compatible with either bacterial encephalitis or eastern equine encephalitis virus (EEEV) infection (Fig. 1a). Tests for rabies virus, West

Nile virus (WNV), and equine herpesvirus 1 (EHV-1) infection were negative. **The rtPCR test for EEEV was strongly positive** on both fresh brain tissue (inactivated by TriPure. Ct 18.09), and on scrolls prepared from formalin-fixed paraffin-embedded tissue (Ct 18.68). Immunohistochemistry was also strongly positive for EEEV antigen (Fig. 1b).

From Aug 1 to Oct 31 of this year, 5 horses (or their brains) with clinical signs of encephalitis have been submitted to the AHL for postmortem examination. Two of these were nonspecific nonsuppurative encephalitis, one was suspicious for *Sarcocystis neurona*, and there were single cases of WNV and of EEEV infection.

Although rare, we tend to think of EEEV as less common than WNV infection of horses, however, **since 2008 we have confirmed more cases of EEEV than WNV or rabies virus** (Table 1) infection in horses.

EEEV is an arbovirus with seasonal incidence, transmitted by mosquitoes from wild birds. Of the 8 pathology cases seen at the AHL since 2008, 1 occurred in August, 6 in September, and 1 in early October. *AHL*

Figure 1. Cerebral cortex of EEEV-infected horse. a) Widespread neutro-

phil-rich encephalitis. H&E. (Close-up in insert).

b) Immunohistochemistry of EEEV demonstrating large amounts of brown-staining viral antigen in neurons (arrows).



Table 1. AHL pathology cases of diagnosed viral encephalitis in horses.

Year	EEEV pathology cases	WNV pathology cases	EHV-1 encephalitis pathology cases	Rabies pathology cases	Total rabies tests sent to CFIA (horses, in- cluding non-pathology cases)*
2008	1	1	1	1	42
2009	1	0	0	0	27
2010	0	0	0	0	19
2011	1	2	2	0	32
2012	0	2	0	0	24
2013	0	0	1	0	20
2014	3	0	0	0	7
2015	1	0	1	0	10
2016	0	0	0	0	15
2017	1	1	0	0	6
Total	8	6	5	1	202

COMPANION ANIMALS

How to prepare diagnostically useful cytology slides! Kristiina Ruotsalo

To maximize the diagnostic value of cytology submissions, it is important to submit **optimally prepared smears** along with a **relevant clinical history**. The goal of slide preparation is to obtain a monolayer of cells which can be adequately stained and evaluated microscopically, without excessive crush artefact or cell lysis. Helpful hints:

- ⇒ Use good quality glass slides with a frosted end to record patient ID and collection site on each slide (e.g., Fluffy Smith, R leg mass). Use a pencil or 'sharpie' marker so that this information is not lost during slide staining.
- ⇒ Have all the materials required close at hand (consider assembling a cytology sample kit) so that no time is lost between sample acquisition and slide preparation. In addition to slides, needles, and syringes, keep a few EDTA and serum tubes close by in case aspiration yields fluid material.
- ⇒ Make sure that there is adequate 'person power' (or a small fan) available so that the slides can be quickly air dried immediately after they are made. Dry the slides briskly (think vigorous 'jazz hands' while firmly grasping the slides), to preserve cellular morphology. Slow drying results in cellular rounding artefact, making it difficult or impossible to assess morphology. Please refrain from blowing on the slides to dry them; this may add moisture to the preparation and distort cellularity. Store air-dried smears in cardboard or foam slide containers to protect them from environmental contaminants, moisture, and excessive temperatures.
- ⇒ Do NOT heat-fix cytology or hematology slide preparations. Avoid exposure to formalin fumes, which will partially fix the cells and alter their morphology.
- ⇒ Fine-needle aspiration (FNA) biopsies are done utilizing a 21-22 ga needle with or without a 5-12 mL syringe, depending upon whether an aspiration procedure or a capillary technique is used. For more detail: *Raskin & Meyer, Canine and Feline Cytology, 2nd ed Valenciano & Cowell, Diagnostic Cytology and Hematology* of the Dog and Cat, 4th ed.

Following FNA, the harvested material needs to be applied onto slides **immediately** so that a monolayer is achieved and there is optimal cellular preservation. Samples containing blood or fluid are best spread using a **blood smear technique** (Fig. 1). Preparation of direct smears from concentrated cell buttons (sediment smears) following fluid centrifugation will maximize the number of cells available for cytologic evaluation while maintaining cellular integrity; this is particularly helpful if there are inherent delays related to sample transit to the laboratory, or for fluids such as urine when submitted for cytologic evaluation.

Semi-solid or solid material obtained by FNA is best

spread using a **'slide-over-slide' technique** (Fig. 2) illustrated below. When used properly, this is generally the best and most reliable method of preparing slides from FNA or scrapings of solid tissue, and my method of choice. The collected material is expelled near one end of the **sample slide**. A **spreader slide** is placed on top of and parallel to the sample slide. The aspirated material will generally spread out between the slides because of the weight of the spreader slide alone, and no additional pressure is typically required. If the sample is thick or granular, very gentle pressure may be applied to the spreader slide but do not 'squash' the slides **together!!** The spreader slide is then lightly drawn across the length of the sample slide. The end result should be 2 slides with material spread into a thin monolayer, and both are suitable for staining and evaluation.

Please do not spread aspirated material onto slides using the needle (i.e., NO 'starfish' preparations)! Similarly, material should not simply be sprayed onto the slides with no additional efforts at spreading the sample. Such preparations typically result in thick smears with numerous ruptured cells and a non-diagnostic sample.

Ulcerated or exudative superficial lesions are amenable to **impression smears**. Remember to remove excess blood or fluid exudate prior to sampling and gently press the slide to the lesion, rolling it off slowly, to ensure that a thin layer of cellular material remains. It is possible that these samples represent only the superficial aspect of the lesion, and further investigation may be required.

Material gathered from **swabs** (e.g., uterine or vaginal) should be gently rolled onto a slide immediately after acquiring the sample to prevent drying of cellular material. Please do not submit the swab to the lab for cytologic preparation as cellular material will have invariably been rendered uninterpretable because of drying. *AHL*





Figure 2. 'Slide-over-slide' technique - do not squash!

Figure 1. Blood smear technique