Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

AHL Newsletter, Volume 25, Number 2 **June 2021**

In this issue:

AHL Newsletter
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The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.
Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends.
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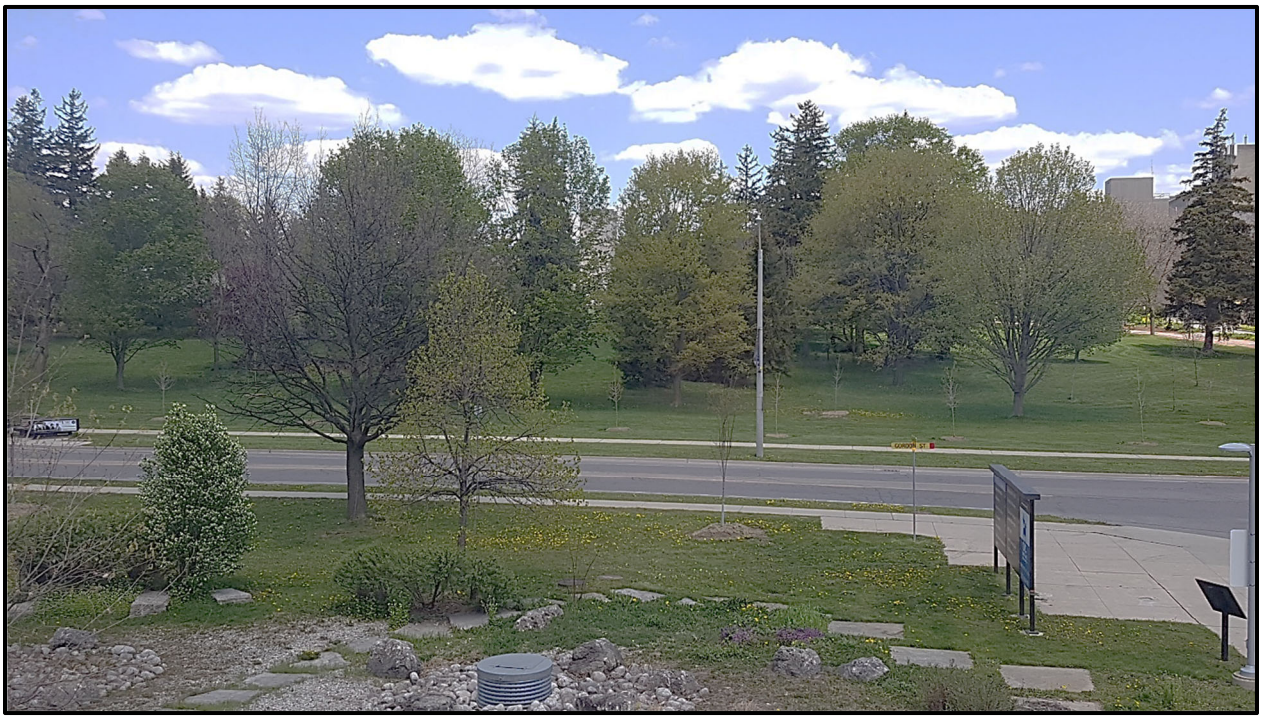
Update from the Director



The view from the Director's office

The weather the past few days has been more summer-like than the spring foliage depicted in the photo above would suggest. Work at the AHL this spring remains busy as usual, and most staff are working on site. We continue to monitor Public Health updates for the best way to protect ourselves from SARS-CoV-2 infection, and have recently upgraded to medical masks (now readily available), as per U of G requirements. Although most AHL staff have received their first vaccine dose, we continue to be very cautious of the high infectivity rate of variants and the risk of developing symptomatic COVID. As a business situated in Guelph, AHL is fortunate to be able to participate in the Guelph Chamber of Commerce's COVID rapid testing program. A voluntary COVID rapid testing clinic is held once a week in Building 89, together with Department of Pathobiology faculty, staff and students. We are also participating in a University of Guelph wastewater testing pilot project, as this technique has shown high sensitivity for identifying infected individuals 5-10 days prior to becoming symptomatic. As COVID case numbers in Ontario decrease, it becomes critical to maintain a high level of testing to detect positive people and conduct contact tracing if we are to emerge from this pandemic. AHL is trying to do our part!

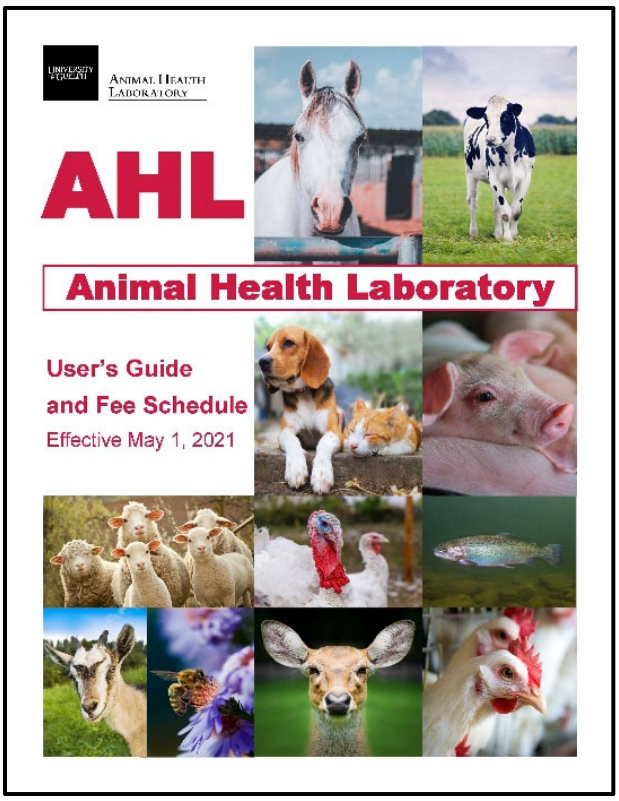
Please check out our 'Staff highlights' section on whole genome sequencing by Dr. Đurđa Slavić, AHL Bacteriologist. Whole genome sequencing (aka next generation sequencing) is a relatively novel technology that has the potential to revolutionize how pathogens such as viruses and bacteria are identified. Although it is currently slower and more expensive to perform than traditional culture and PCR, future modifications will no doubt render it more useful in a diagnostic setting.

For our equine practitioners, we now have PCR panels available for foal diarrhea and adult diarrhea testing; an equine respiratory panel is also in development. Client feedback has indicated that panels are highly desirable to assist in diagnostic test selection, and we strive to meet this request.

From all of us at AHL, we wish you a safe and healthy summer, with time off to relax with your families and friends.

Maria Spinato, Director

Animal Health Laboratory, University of Guelph, Guelph, ON.



Includes test information, new tests, new test panels, and more!

Mobile friendly!

Available on-line at https://www.uoguelph.ca/ahl/

Test information is linked to LabNotes to facilitate test selection and interpretation of results.

New tests since May 2020:

- *Anaplasma marginale* and *A. centrale* duplex qPCR
- *Renibacterium salmoninarum* qPCR (Bacterial Kidney Disease)
- Swine dystrophin genotyping
- African Swine Fever PCR Surveillance
- Rotavirus, group A,B,C – Sequencing
- Avian metapneumovirus (AMPV) – ELISA
- Ureaplasma culture, semen
- Bacterial count, total viable in fish feces
- Bovine comprehensive respiratory panel
- *Cryptosporidium* species PCR
- Bovine astrovirus – PCR
- Hatchery - excess mortality - *Salmonella* culture
- Hatchery, reactor - *Salmonella* culture
- Whole Genome Sequencing
- *Campylobacter fetus* sbsp *venerealis* – Bacterial culture
- *Salmonella* Dublin – qPCR.

Online fee schedule

Please note: The fee schedule is available only to veterinarians, veterinary organizations, and researchers. We have changed the login and all clients must now have a unique username and password.

Please visit https://www.uoguelph.ca/ahl/user/register to register. (Internal University of Guelph clients – please email ahlinfo@uoguelph.ca for set up.)

Questions? ahlinfo@uoguelph.ca (519) 824-4120 ext 54530.

AHL Specimen Reception update

We hope everyone is enjoying the warmer weather. Summer brings extra precautions when shipping parcels with perishable diagnostic specimens, so don't forget those ice packs!

The vestibule at AHL Guelph is available from 7AM to 10PM every day for drop-off of submissions.

Please remember that samples dropped off **must be packaged to prevent leakage** similar to sending by courier! And also remember to **include a fully completed submission form**! We don't like to play detective!

On our main AHL website page are 4 videos – a virtual tour of the lab and 3 videos related to filling out the submission form, packaging and splitting and preparing samples. Please have a look!

https://www.uoguelph.ca/ahl/

Here are some pertinent points to consider during the summer months. Although the couriers are somewhat more reliable now, there are still times when submissions can be delayed. There will be concerns around critical temperature-sensitive testing unless extra precautions are taken.

From our Labnote 27: https://www.uoguelph.ca/ahl/submissions/ahl-labnote-27-submission-instructions

- Separate serum from clot after complete blood clotting has taken place.
- Always include a blood smear for all complete blood count (CBC) samples.
- Use styrofoam insulating containers and freezer packs (**Fig. 1**).
- Wrap samples in insulating material (e.g. paper).
- Ship earlier in the week to avoid hold-overs on the weekend.

Figure 1. Styrofoam container, ice packs and packaging material for optimal transport of lab submissions.

Thanks very much for your patience and understanding.
ahlinfo@uoguelph.ca (519) 824-4120 ext 54530.
Jim Fairles, Jennifer Zoethout, Client Services and Specimen Reception.

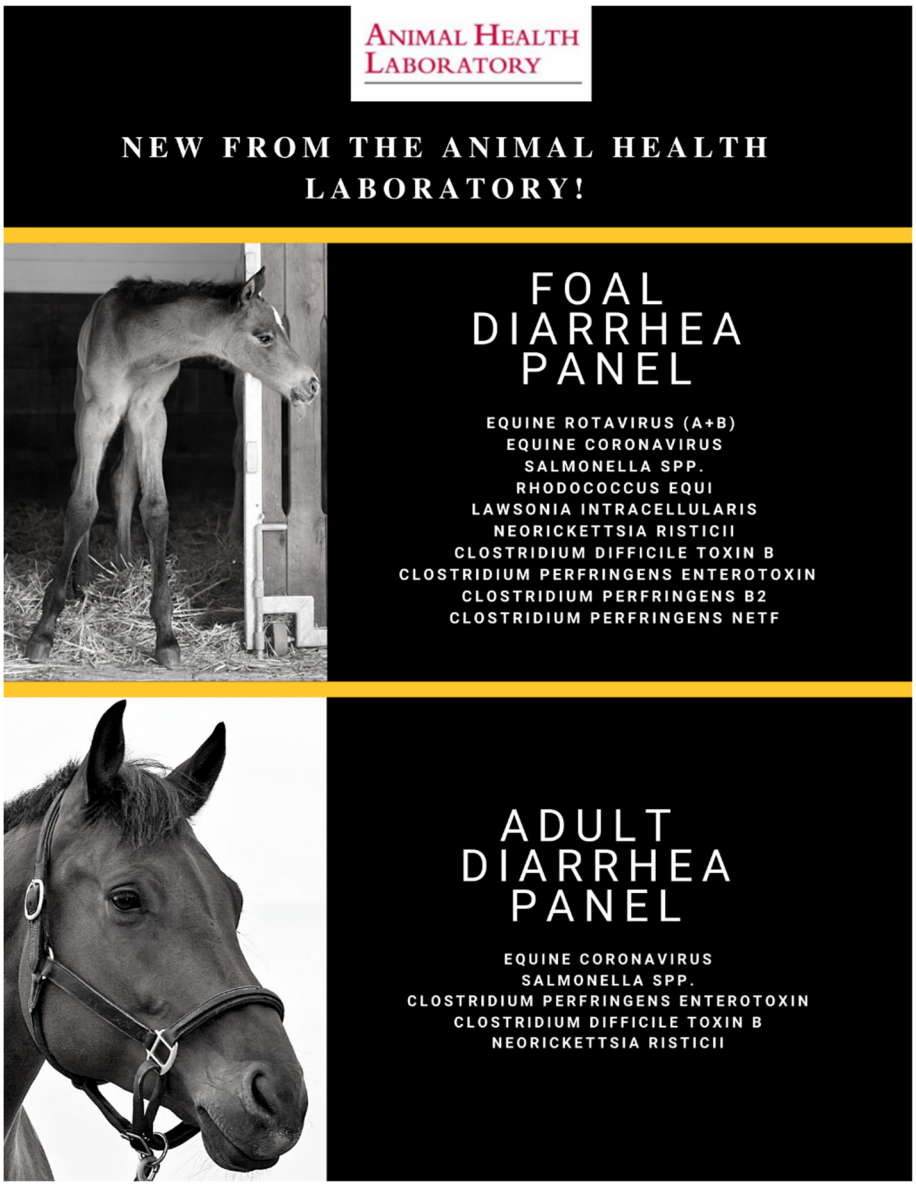
Equine diarrhea PCR panels

Jim Fairles, Melanie Barham

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):5.

In response to client demand, we are pleased to offer two new panels for diagnostic testing of equine diarrhea pathogens: a foal panel for horses less than a year of age, and an adult panel for horses more than a year of age. Developed specifically for Ontario horses, our new panels are affordable and customized for the needs of Ontario veterinarians and their herds.
To launch the panels, we are offering **free** testing until June 30, 2021 to our Ontario clinics. If you wish to submit a free sample, please send in 15 g of feces and code "foal diarrhea panel" or "adult equine diarrhea panel" on the submission form. After June 30, please contact the lab for pricing of panels. *AHL*



Staff highlights: Whole genome sequencing (WGS) at AHL

Đurđa Slavić

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):6.

Whole genome sequencing (WGS), also called next generation sequencing (NGS), is a very powerful technology that provides a large amount of sequencing data cost-effectively and in a relatively short period of time. It is also known as massively parallel sequencing because sequencing of multiple samples can be done at the same time.

There are numerous clinical applications of WGS. WGS can be used for identification and characterization of novel pathogens (i.e., bacteria, viruses, parasites) in clinical samples, metagenomics, strain typing, susceptibility prediction, and virulence factor determination, among other applications.

In 2019, AHL became the only Canadian core lab for the Federal Drug Administration (FDA) Veterinary Laboratory Investigation and Response Network (Vet-LIRN) that performs whole genome sequencing of *Escherichia coli*, *Staphylococcus pseudintermedius*, and *Salmonella* spp. and deposits sequence data at the National Center for Biotechnology Information (NCBI). To date, more than 300 bacterial isolates have been sequenced in support of the Vet-LIRN program which monitors antimicrobial resistance trends in bacteria isolated predominantly from dogs. This trend will continue in upcoming years with an estimated 300 bacterial isolates being sequenced annually.

In addition to the Vet-LIRN project, AHL has been doing WGS of other clinical bacterial isolates (i.e., pure culture) to gather additional information. For the Ontario Animal Health Network (OAHN) swine network, AHL performed WGS of *Erysipelothrix rhusiopathiae* isolates from Ontario. Based on WGS data, we were able to determine antimicrobial resistance determinants and virulence factors. Moreover, this work showed that there are 8 different multilocus sequence types (MLST) present among Ontario isolates. Only one belonged to the previously described sequence type (ST), whereas seven STs were not previously known (1).

To establish the origin of same *Salmonella* serotypes, AHL did WGS of 30 *Salmonella* isolates as well. Based on whole genome MLST data, it was possible to determine which isolates originated from the same source and which did not, a valuable tool in tracing outbreaks. AHL used WGS to detect virulence factors and to determine the O and H types of *E. coli* isolated from poultry. WGS was also instrumental in confirming that *Streptococcus equi* subsp. *zooepidemicus* isolated from a recent swine case belonged to the more virulent ST194 strain known to cause disease outbreaks and mortalities in swine operations in Manitoba and the USA (2).

Additional characterization of isolates may also have significant clinical application when it comes to vaccine production, as was shown in one of our cases. When vaccine isolates were compared to clinical isolates it was shown that they belong to different MLST types, explaining the clinical outbreaks of disease despite routine vaccination.

These are just a few examples of the utility of WGS in clinical applications. At present, AHL offers WGS of bacterial isolates to obtain relatedness and virulence information, as specified above. We are also working to expand WGS to include the detection of bacterial and viral pathogens directly from clinical samples. *AHL*

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2. DeLay J, et al. *Strepcococcus equi* subsp. *zooepidemicus* septicemia: First confirmed case in Ontario swine. AHL Newsletter 2021; 25(1):11.

Bacterial taxonomy update

Đurđa Slavić, Sarah Lippert, Charles Doekes

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):7.

In the next few weeks, the AHL bacteriology section will be updating our bacterial taxonomy list and the following bacterial name changes will be seen on the AHL reports:

Old name	New name
Haemophilus parasuis	*Glaesserella parasuis*
Clostridium difficile	*Clostridioides difficile*
Clostridium sordellii	*Peniclostridium sordellii*

AHL

Update on submission of live animals for postmortem

Andrew Brooks

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):7.

The global shortage of pentobarbital has impacted the ability of the AHL to accept live animal submissions. We are no longer able to source supplies of euthanasia solution comprised of this chemical. Whenever possible, please ensure that animals are humanely euthanized and submitted for postmortem as soon as possible. **Although live food animals can still be submitted, we request that you please contact the AHL in advance in order to confirm that the submission can be accepted.**

Live poultry submissions are not affected as pentobarbital is not required for humane euthanasia of birds. *AHL*

Changes in AHL Histopathology charging parameters for 2021/2022

Josepha DeLay, Susan Lapos, Jim Fairles Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):7.

Please be aware of several changes to several AHL histopathology tests, beginning on May 1, 2021.

- Food animal histopathology cases with >25 pieces of fixed tissue submitted will have additional charges applied. This is to cover additional technical and pathologist time required to trim, process, and interpret a large number of samples. The additional charges will include either doubling of the histopathology test fee, or a per slide fee if >10 slides, depending on the specific case.
- Additional fees for decalcification or nail / hoof softening will be applied to all cases requiring these extra procedures to prepare tissues for histologic sectioning.
- For excisional tumor biopsies >2 cm diameter, margin evaluation will only be done if requested on the submission form. Additional charges will apply. *AHL*

OAHN update – June 2021

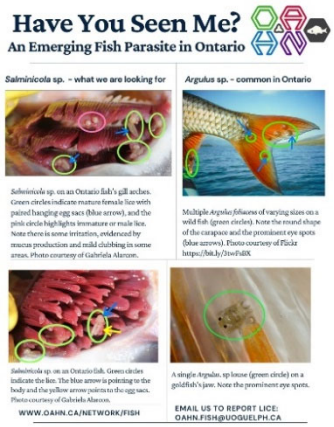
Mike Deane and Melanie Barham

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):8.

The Ontario Animal Health Network has been busy throughout the winter and spring, with the completion of many research projects, new info sheets, reports, and a disease alerts page. Read on to find links to all the exciting animal health and disease research and resources we have been working on.

New OAHN Resources



Have You Seen Me? An Emerging Fish Parasite in Ontario

The OAHN Fish Network has created a valuable resource for identifying fish lice in Ontario. You can access the resource here: https://www.oahn.ca/resources/an-emerging-fish-parasite-in-ontario/

Ontario Equine Disease Alerts Page

The OAHN Equine Network has created a new page in the OAHN website that contains up-to-date Ontario equine disease information. This resource is for both veterinarians and owners, and gives details on all known cases since the spring. Access this page here: https://www.oahn.ca/resources/ontario-equine-disease-alerts/

OAHN Project Reports

This spring saw the completion of many OAHN-funded projects. We currently have many other projects on the go, including this project on SARS-CoV-2 in pets. Find details and links below for recently completed projects:

- OAHN Swine Network Research Project: Modelling potential hotspots of African Swine Fever in Ontario's wild pig population
- OAHN Bovine Project: Parasitism in Grazing Cattle in Ontario
- OAHN Small Ruminant Research Project: Development and validation of a PCR test for small ruminant lentiviruses (CAEV and MVV)
- OAHN Swine Project: Ongoing investigation of an outbreak of Senecavirus A
- OAHN Bovine Project: Surveillance of postmortem data from a livestock disposal site
- Aquatic Network Research Project: Preliminary investigation into a proprietary feed-based probiotic to reduce natural coldwater disease in rainbow trout aquaculture in Ontario

2 New Bovine Podcasts

The OAHN Bovine Network created two new podcasts for Ontario bovine veterinarians and producers:

- Parasitism in Grazing Cattle in Ontario with Dr. Jessica Gordon
- OAHN Bovine Network: Quarterly Update April 2021

New Reports and Resources

The latest network reports for companion animals, bovine, swine, poultry, and aquatic animals have been posted to the OAHN site under "Network Reports". Be sure to check out **OAHN.ca**!

Congenital disproportionate dwarfism in a beef calf

Margaret Stalker, Gabriel Jantzi

Animal Health Laboratory, University of Guelph, Guelph, ON (Stalker), Metzger Veterinary Services (Jantzi)

AHL Newsletter 2021;25(2):9.

A one day-old cross-bred beef calf was submitted to the AHL for postmortem examination. The calf came from a small herd of cross-bred and Hereford cows with a Simmental bull, with static genetics. Of 20 currently calved this spring, 10 calves were clinically affected by a form of congenital dwarfism.

On postmortem examination, the calf was small statured (Fig. 1), with disproportionate shortening of all four limbs relative to the body and enlarged-appearing joints (Fig. 2). No arthrogryposis or abnormalities of the head or spinal column were noted. Histologic examination revealed reduced thickness of the physeal zones of hypertrophy and provisional calcification, with premature physeal closure in some joints, compatible with a chondrodystrophy. Liver trace mineral analysis revealed a manganese level of 1.0 ug/g (reference interval 2-6 ug/g), and a zinc level of 140 ug/g (reference interval 24-100 ug/g). PCR testing for BVDV was negative.

Differential diagnosis of congenital short stature in livestock includes genetic chondrodysplasia, nutritional chondrodysplasia, and chondrodysplasia due to exposure to plant toxins or toxic levels of vitamin A:

- *Genetic chondrodysplasia* typically has a recessive inheritance so only a small number of animals are expected to be affected in the herd. Affected animals are relatively uniform in their physical presentation with shortened long bones, enlarged joint epiphyses and often a domed head with brachygnathia inferior.
- *Nutritional chondrodysplasia* is associated with low liver zinc or manganese concentrations, although this may be a transient change if deficiency occurred for a finite period during gestation. This form of chondrodysplasia typically has a higher prevalence in the herd than expected for inherited forms of chondrodysplasia. Affected animals may vary in the severity of dwarfism, and animals with mild cases may improve after birth. Again, shortened long bones and enlarged epiphyses are typical, although the head abnormalities are not. Histologic changes of genetic and nutritional chondrodysplasia are very similar, with alterations in thickness of the physes.
- *Plant toxins* are not typically a cause of chondrodysplasia in calves in Ontario. The diagnosis of "acorn calves" has been used historically, although cases were found to be associated with a more general maternal nutritional deficiency (see above) rather than specific exposure to acorns. Maternal exposure to *Veratrum californicum* (white false hellebore) is the classical cause of congenital malformations including cranial and limb deformities in sheep and cattle in some areas of the world. This plant species is not present in Ontario although a related species, *Veratrum viride* is present in Quebec. Ingestion of wild lupines can also cause shortening and rotation of long bones and flexural contractures ("crooked-calf disease"); but again, wild lupines (*Lupinus spp*.) are not widespread in Ontario.
- *Vitamin A toxicity* due to supplemental injection of newborn calves has been associated with an unusual form of dwarfism with premature closure of the growth plates of the pelvic limbs resulting in a sloping back and small hindquarters, so-called "hyena disease".

In this case, given the genetics of the herd, type of limb deformity and high prevalence, an underlying nutritional cause was suspected. Evidence to support this however can be difficult to obtain. The

literature suggests this form of chondrodystrophy may be associated with a maternal deficiency of manganese or zinc during a critical stage of gestation for limb development. This calf had liver manganese levels below the reference interval (RI), however the RIs were developed for adult animals, and calves are normally born with lower levels of liver manganese which increase over the first few weeks of life. Interestingly, the literature states that some affected calves can be born with completely normal levels of liver trace minerals, possibly reflecting the earlier time period of the insult.

There is an association with grazing drought-affected pastures, or feeding silage/fermented forages to spring-calving cows during mid- to late-gestation, typically without dry feed or grain supplementation. Although outright deficiency is possible in some diets, it has also been suggested that the bioavailability of manganese may be reduced in some ensiled forages, or that alterations in other minerals or factors may contribute to the necessary conditions to produce the long bone deformities. In this case, this herd was being fed oat and pea silage, one of two cuts of sorghum, and a commercial beef premix. Feed analysis of the silage and sorghum had mineral levels, including manganese and zinc, within the expected range on a dry matter basis. The premix was initially fed free choice, then restricted to 100g/head/day and top-dressed. Actual intake per cow is more difficult to assess.

The frequency of congenital defects such as this in Ontario cow-calf operations is unknown. Gross postmortems to document deformities and further categorize these cases may yield useful information for producers, and may inform future discussions on optimizing mineral delivery to beef cows throughout pregnancy. *AHL*

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1. Dittmer KE, Thompson KG. Approach to investigating congenital skeletal abnormalities in livestock. Vet Pathol 2015;52:851-861.
2. Buskirk D. 2015. Prevent calf abnormalities by managing beef cow diets. Michigan State University Extension.
3. Proulx JG, Ribble CS. 1992. Congenital joint laxity and dwarfism in a beef research herd. Can Vet J 33: 129-130.

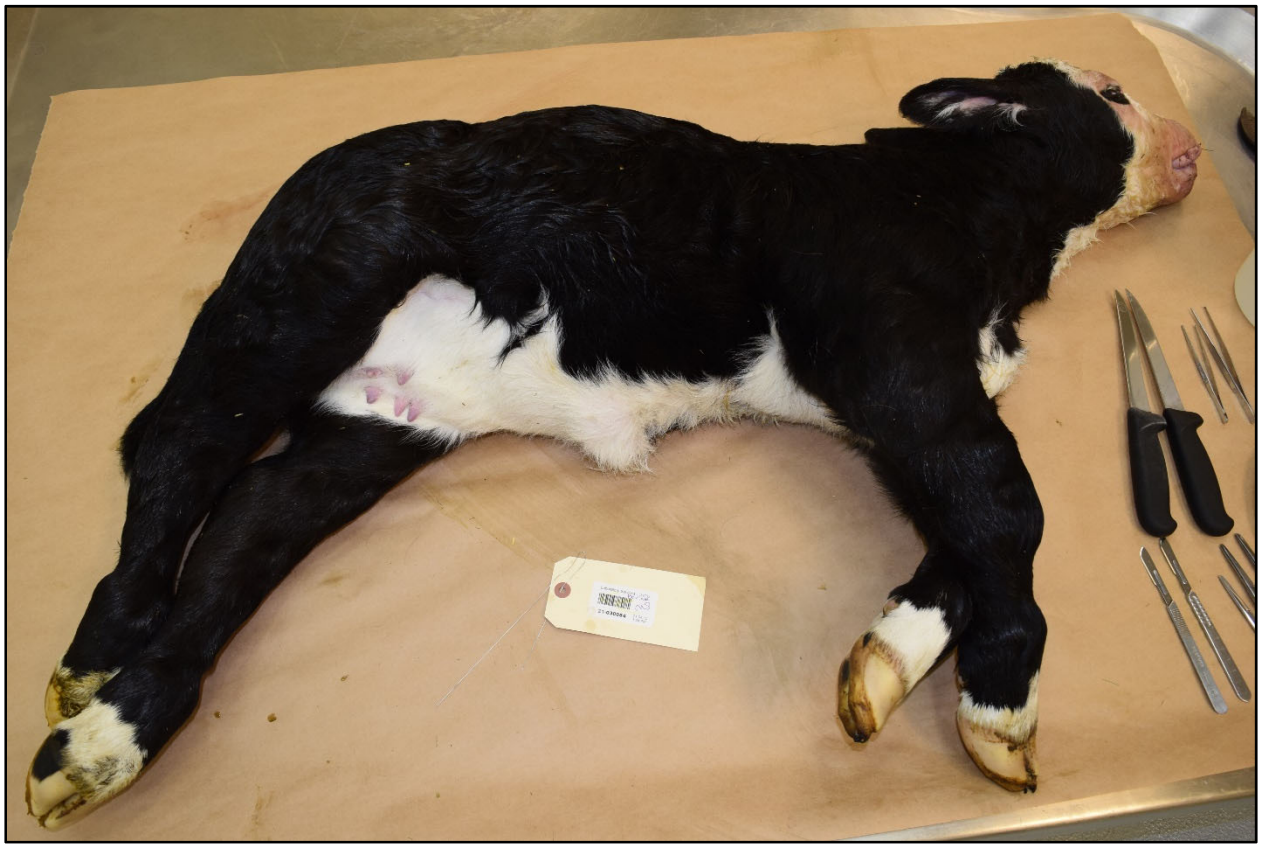

Figure 1: One day old beef calf with shortened limbs and enlarged joints (most apparent in the forelimbs).

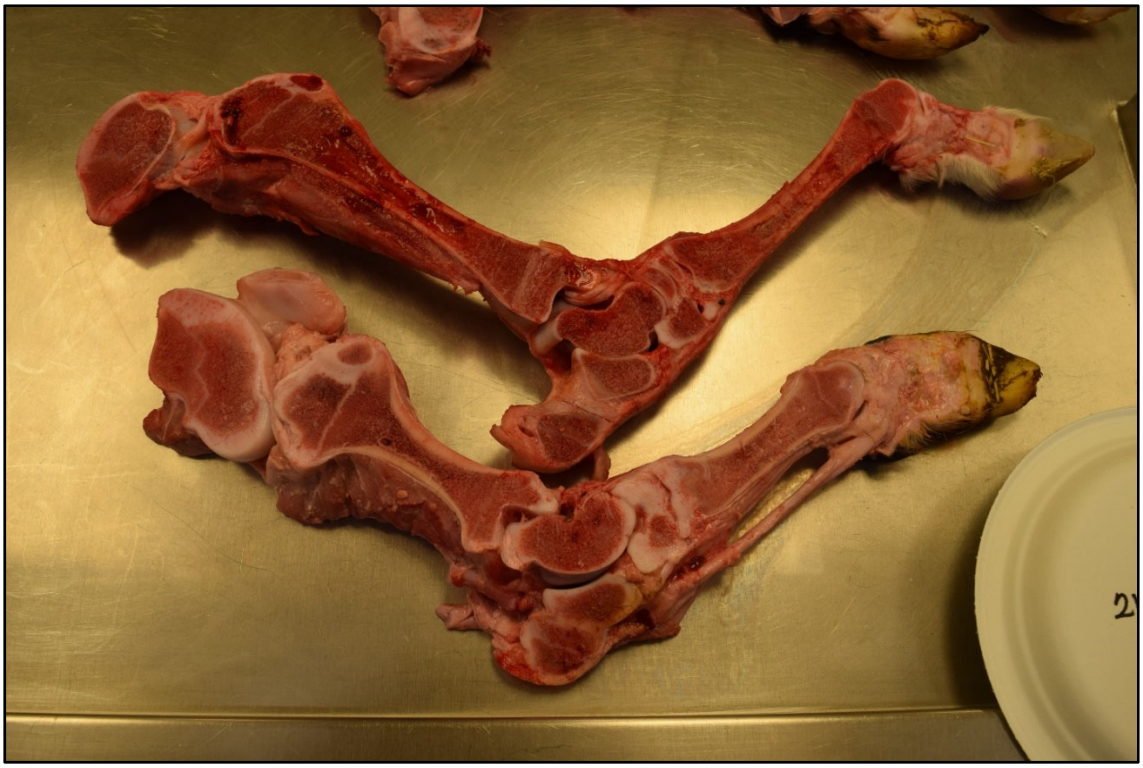

Figure 2: Sagittal sections of distal hind limbs: top limb is an age-matched Holstein calf for comparison; bottom limb is an affected beef calf showing the shortened tibia, thickened cortex and enlarged epiphyses.

Congenital BVDV type 1 infection in a group of lambs: a shaky situation

Rebecca Egan, Davor Ojkic, Jim Fairles, John Van Ostaaijen, Jocelyn Jansen

Animal Health Laboratory, University of Guelph, Guelph, ON (Egan, Ojkic, Fairles), Eldale Veterinary Clinic (Van Ostaaijen), OMAFRA (Jansen).

AHL Newsletter 2021;25(2):11.

Over a two-week period, an Ontario sheep flock had approximately 10 lambs born with tremors and weakness (see video link below), and a diagnostic investigation was carried out to investigate the cause. Clinically, the tremors seemed to worsen when the lambs were excited, and while some of the stronger lambs were able to nurse, weaker lambs succumbed to the disease. In the month leading up to lambing, the pregnant ewes had been sheared, deloused and vaccinated against clostridial diseases, and the dams were generally in good health leading up to and following lambing. An important piece of clinical history was provided as well: young Holstein calves sourced from various regions of Ontario and Quebec were being brought in and raised at this farm, and these animals were not vaccinated against BVDV prior to arrival. In addition to sharing airspace in the barn, some calves were housed in a pen directly beside a group of pregnant ewes. There was a solid cement partition wall with a shared waterer, which could have been a source of viral transmission via saliva. Additionally, there was potential transmission via the movement of staff and equipment.

CLICK THE LINK TO WATCH THE VIDEO! → https://youtu.be/Yibhn1zQjBs

An affected 1-day-old lamb was submitted to the AHL for postmortem examination. The lamb was in thin body condition with partial depletion of fat stores. Subtle changes were observed in the brain. In the cerebral cortex, the interface between the grey and white matter of gyri appeared slightly darker than usual, and the adjacent white matter tracts were slender. The cerebellum appeared normal in size with equivocal flattening of folia noted in some areas. Congenital copper deficiency (swayback) can cause similar neurologic signs stemming from lesions in the white matter of the CNS, and therefore, a liver sample was submitted for assessment of copper level which was found to be sufficient. Histologically, the distinction between grey and white matter in the brain and spinal cord was less well-defined than normal, the white matter tracts were slender (compatible with hypomyelination, which could be highlighted with a special Luxol fast blue stain), and there was prominent gliosis. Immunohistochemistry was pursued and identified immunoreactivity for bovine viral diarrhea virus (BVDV) in multiple organs. These included brain, intestine, urinary bladder, spleen, and liver, with involvement of multiple tissue elements such as neurons, glia, mural ganglia, epithelial cells, vascular smooth muscle, and interstitial macrophages/dendritic cells (**Fig. 1**). The abundance of viral antigen and immunohistochemical staining pattern supported persistent viral infection in this lamb. It is important to note that anti-BVDV antibody will also recognize Border Disease virus antigen, a closely related pestivirus; however, in this case, PCR testing of spleen and ileum detected BVDV type 1.

In sheep, infection with Border disease virus (BDV) can result in abortions, stillbirths, and small weak-born lambs, some of which may have *arthrogryposis, mandibular brachygnathism, thymic hypoplasia*, and hairy fleece and tremors (known as 'hairy-shaker' lambs), with the latter stemming from CNS lesions. This virus is very closely related to BVDV, with both viruses belonging to the *Flaviviridae* family, thus it is not surprising that infection of sheep by BVDV types 1 or 2 are capable of producing Border Disease syndrome in sheep.

Ruminants persistently infected (PI) with BVDV shed the virus and are the main source of spread among herds. A recent study showed that in addition to direct contact, BVDV transmission may occurs as a result of aerosol transmission as well (3). In the current case, BVDV screening was pursued to identify and remove infected animals, and implementation of additional biosecurity measures were recommended to prevent similar cross-species transmission in the future. BVDV screening can be done via PCR testing (EDTA blood or serum, tissues) which will identify both acutely infected and PI animals. Serum samples can be pooled into groups of 5, but samples should be submitted individually so that animals can be tested individually in the event that the pooled sample is positive. Furthermore, a positive PCR result on a single sample cannot distinguish between an acutely infected animal and a PI animal, so positive animals must be re-tested at least 3 weeks following the initial test, at which point PI animals will still have detectable virus and animals that have recovered from acute infection should not. For more information, see AHL LabNote 1 Summary of bovine viral diarrhea virus (BVDV) testing at the AHL. *AHL*

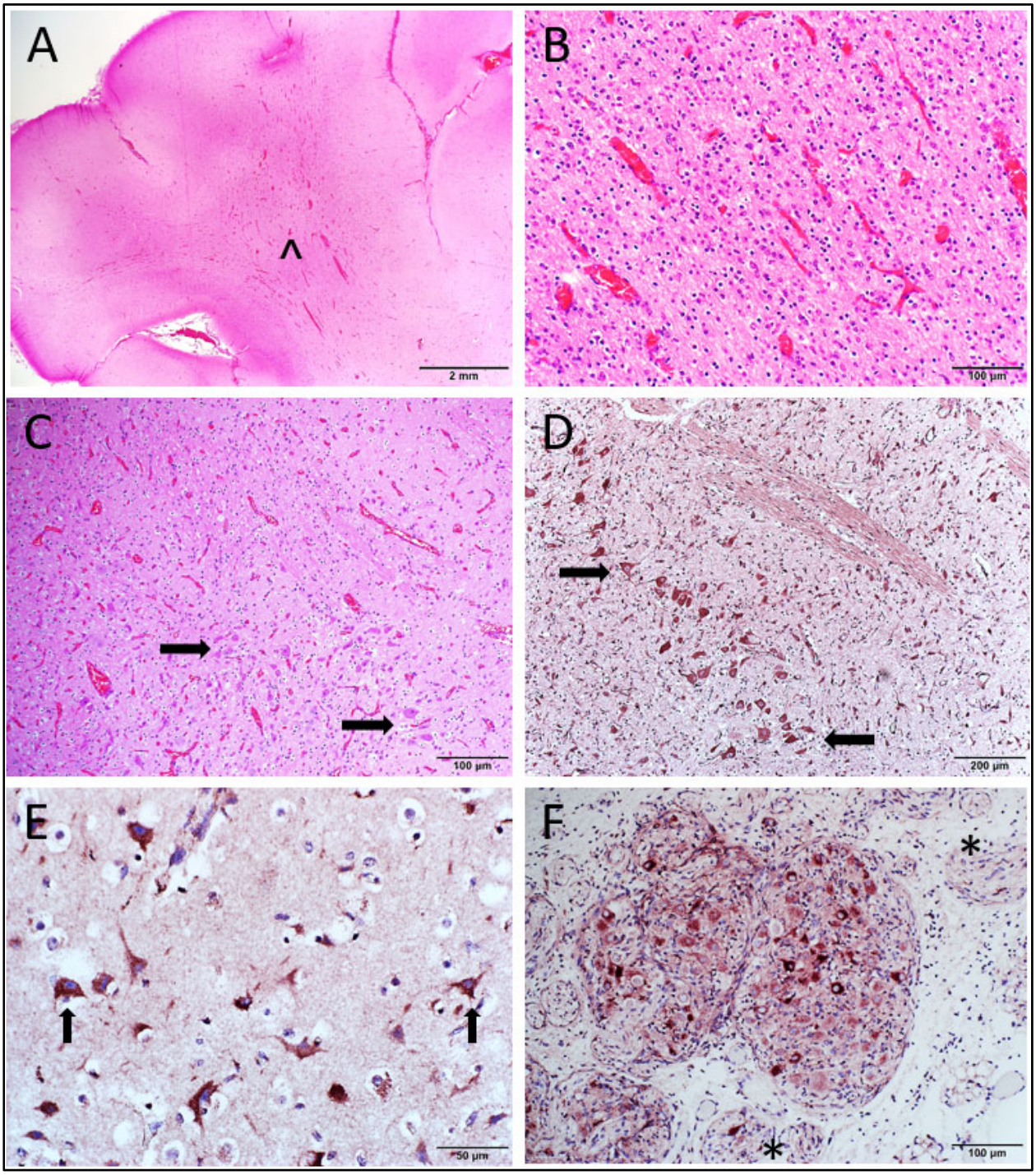

Figure 1. Congenital BVDV type 1 infection in a lamb. Cerebral cortex (A-C) with white matter that displays reduced myelination (A - arrowhead) and gliosis (B). Neuron cell bodies are indicated (C - arrows). H&E stain. Positive immunostaining for BVDV in brain (D-F): neuron cell bodies (D,E - arrows), ganglia (F) and peripheral nerves (*). IHC

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Porcine circovirus 3 (PCV3) results at the AHL, 2018-2021

Josepha DeLay, Davor Ojkic, Jim Fairles

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2021;25(2):14.

Porcine circovirus 3 (PCV3) was first identified in 2015 in association with reproductive loss and maternal systemic disease in a sow herd in the United States. Systemic disease in growing pigs has also been linked to PCV3 infection. Retrospective studies have confirmed that PCV3 has been present but undetected in swine herds for many years, and association with clinical disease is a recent event. Direct disease causation by the virus remains to be proven, and Koch's postulates have not yet been fulfilled, although ongoing research has provided evidence of disease in swine that is linked to presence of the virus.

In sow herds, PCV3 has been associated with an increase in mummified and stillborn fetuses, and with weak neonates. Litters from lower parity sows are most commonly affected. Poor growth in older pigs has also been associated with PCV3. Histologic lesions in in PCV3 infected fetuses and pigs include lymphocytic myocarditis and myocardial fibrosis, and lymphocytic perivasculitis in various organs, especially in heart and kidney. Using *in situ* hybridization (ISH) methods, PCV3 nucleic acid can be detected in tissues, co-localizing with histologic lesions. Immunohistochemical (IHC) assays for PCV3 currently have very limited availability and are restricted to research use.

Polymerase chain reaction (PCR) testing for PCV3 began at the AHL in 2018. The cycle threshold (Ct) value for PCR is important in interpreting the significance of the results and provides an indication of the viral load in the sample. Subjectively, PCV3 Ct values of approximately 20 or less are considered significant and potentially associated with clinical disease, if the clinical scenario and histopathologic findings are compatible with PCV3-associated disease. Importantly, a PCV3 Ct value of ≤20 is <u>not</u> a validated cutoff value; rather, this is a guide for interpreting the potential significance of the results. Higher Ct values may be clinically significant in some situations, depending on microscopic lesions, clinical history, and the stage of infection. Alternatively, higher PCV3 Ct values (representative of lower viral load) may not be directly associated with disease and may only indicate PCV3 presence in the herd. Consideration of clinical and pathologic context is important in the interpretation of PCV3 PCR test results.

Between December 2018 and May 2021, the AHL carried out PCV3 PCR tests on a total of 845 samples, representing 278 cases (**Fig. 1**). PCV3 nucleic acid was detected in 261 samples (31%) and in 84 cases (30%). PCV3 Ct values were ≤20 and considered likely clinically significant in 13 / 845 (1.5%) samples and 12 / 278 (4.3%) cases. Significant individual PCV3 PCR Ct values ranged from 16.63 to 20.97, and represented 10 farms. Although the overall proportion of cases in which PCV3 was detected was high (30%), the viral load was considered to be potentially clinically significant (low Ct) in relatively few cases (4.3%).

Useful samples for PCV3 PCR testing include pooled tissues (lung, heart, kidney) from fetuses or older pigs, fetal thoracic fluid, and processing or castration fluids.

Among AHL pathology cases between 2018 and May 2021, correlation of PCR results with histologic lesions led to a diagnosis of PCV3-associated disease in the following clinical scenarios:

- reproductive loss due to increased stillborn and mummified fetuses and / or weak neonates (6 cases)
- neonates with a complaint of failure to thrive (3 cases)
- nursery (1 case) or grow-finish pigs (2 cases) with poor growth and ill-thrift

Significant gross lesions were not detected in any of the cases. Demonstration of specific histologic lesions was important in making an association with PCV3 infection. These lesions included lymphocytic myocarditis and myocardial interstitial fibrosis in fetuses and neonates (**Fig. 2**), and lymphocytic periarteritis in older pigs. PCV3 ISH was pursued in 2 related cases, and PCV3 nucleic acid co-localized with myocardial lesions. PCV3 PCR Ct values for at least 1 tissue sample in all cases were ≤20. Importantly, in cases involving fetuses, PCV3 was not detected in tissue pools from all litters, illustrating the importance of testing fetuses from multiple litters in order to reach a diagnosis involving PCV3 or other viral pathogens. The heterogeneity of PCV3 results also suggests that other pathogens may also be contributing to fetal loss in some cases.

Other lesions that have been described in association with PCV3 infection include non-suppurative encephalitis in neonates, and lesions compatible with porcine dermatopathy-nephropathy syndrome (PDNS). These manifestations of PCV3-associated disease have not been identified to date in Ontario swine.

Although PCV3 belongs to the same virus family as PCV2, vaccines do not provide cross-protection between the 2 viruses, and tests for each virus are distinct and will not cross-react. *AHL*

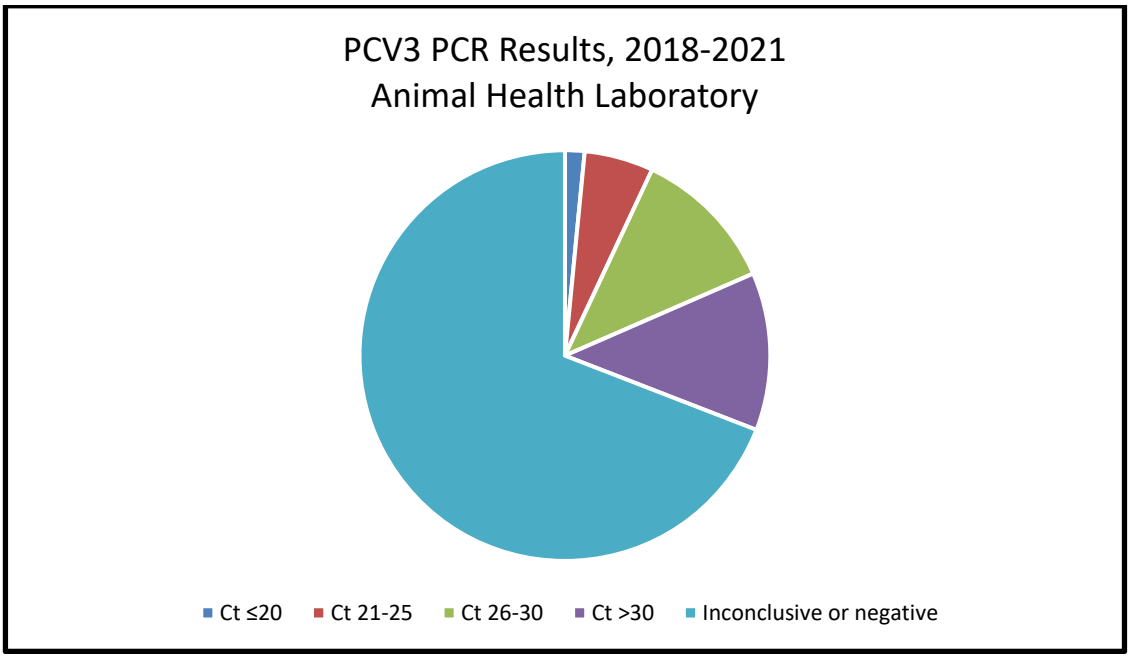

Figure 1. PCV3 PCR results and distribution of crossing threshold (Ct) values for positive cases, 2018-2021. Notably, PCR-positive cases with a low (subjectively significant) Ct value of ≤20 involve a small proportion of cases.

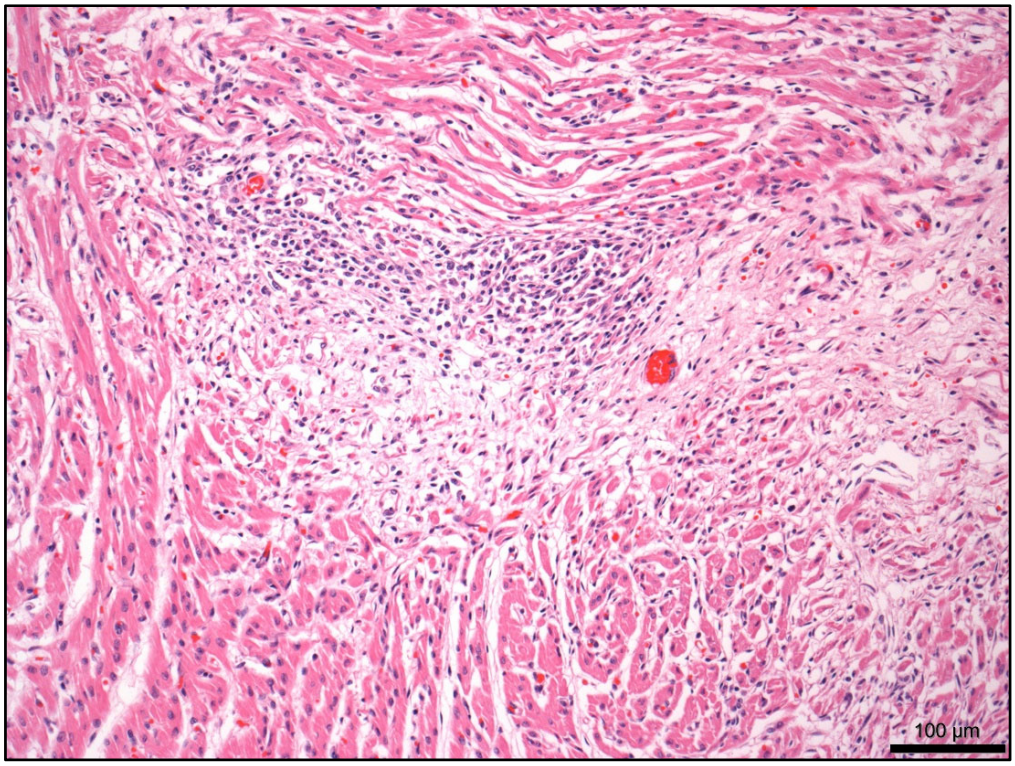

Figure 2. Fetal myocardium, stillbirth associated with PCV3: Cardiomyocyte loss and replacement by fibrosis and lymphocyte clusters. Hematoxylin and eosin stain (10x).

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CanSpot ASF surveillance testing – reminder for swine veterinarians

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AHL Newsletter 2021;25(2):16.

The Animal Health Laboratory continues to test appropriate swine cases under the CanSpot ASF surveillance program. Eligible cases are those for which African Swine Fever is not a differential diagnosis, but have features of specific endemic diseases that could potentially mask more definitive ASF lesions or clinical signs.

To qualify for surveillance testing, cases must have herd location information available (PID or physical address); have appropriate samples available for testing; and meet specific disease criteria listed below.

For cases meeting these criteria, AHL pathologists communicate with and obtain permission from submitting veterinarians prior to surveillance testing. Veterinarians may also initiate testing through communication with the case pathologist or diagnostician.

To facilitate the success of this important ASF surveillance tool, veterinarians can help by:

- Including required fresh tissue samples (i.e. spleen) with field postmortem cases sent to the lab
- Including a thorough clinical history with each case
- Ensuring that the herd PID or physical address is included
- Responding to pathologists' requests for permission for surveillance testing OR provide a signed agreement permitting testing of all eligible cases

Appropriate samples for CanSpot testing:

- spleen, tonsil, kidney, lymph node, terminal ileum, serum

Clinicopathological presentations eligible for CanSpot ASF testing:

1. Septicemia and / or multiorgan hemorrhage such as caused by *E. rhusiopathiae*; *S. suis*; *S. zooepidermicus*; *A. suis*; *S.* Cholerasuis; other bacteria
2. Porcine Reproductive and Respiratory Syndrome virus (PRRS), especially when it causes cyanotic skin
3. Porcine dermatitis and nephropathy syndrome (PDNS) and vasculitis that can be caused by PCV2, PCV3, and other pathogens
4. Hemorrhagic diarrhea / necrotizing enterocolitis such as caused by *Salmonella* spp.; *L. intracellularis; B. hyodysenteriae; B. hampsonii*
5. Fibrinous pleuritis / pericarditis / hydropericardium such as caused by *H. parasuis* (now *G. parasuis*), *S. suis*
6. Mulberry heart disease
7. Splenic torsion
8. Abortion above historical trend for herd

Please contact the AHL or your case pathologist with any questions about the CanSpot ASF program. Thank you for contributing to enhanced ASF surveillance. *AHL*

See also: https://www.uoguelph.ca/ahl/ontario-rolls-out-canspot-asf-enhanced-surveillance-pilot

Disseminated idiopathic myofasciitis in a ferret

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AHL Newsletter 2021;25(2):18.

Formalin-fixed tissues were submitted to the AHL from an 8-month-old, spayed female ferret that presented with a history of mobility problems, weight loss, lethargy and weakness. Severe leukocytosis and neutrophilia were evident in the complete blood count. Disseminated idiopathic myofasciitis (DIM) was suspected clinically and the ferret was humanely euthanized.

Histopathology revealed marked areas of suppurative to pyogranulomatous inflammation in skeletal muscle **(Fig. 1)**, including muscle tissue in the tongue and esophagus. The inflammation consisted mostly of neutrophils with fewer macrophages, lymphocytes, plasma cells and rare eosinophils. There was myofibre atrophy and loss of myofibres within the lesions. The inflammation in the muscular wall of the esophagus was particularly prominent **(Fig. 2)**. Other histological findings included extramedullary hematopoiesis in the spleen and reactive lymphoid hyperplasia in the lymph nodes. In the absence of discernible infectious agents, the morphological diagnosis of multifocal suppurative to pyogranulomatous myositis supported the clinical diagnosis of DIM.

DIM in ferrets is an idiopathic disease with a poor prognosis. An immune-mediated pathogenesis is suspected. DIM usually has a rapid clinical progression and affects young adult ferrets of both sexes, often under 18 months of age. Clinical signs often include weakness, lethargy, fever, depression, anorexia and there is frequently a moderate to marked neutrophilia. Clinical chemistry is not a reliable diagnostic test for DIM since muscle enzymes, such as CK, may not be elevated. At postmortem, the gross lesions may include discoloration the skeletal muscle and muscle atrophy. The esophagus may be dilated, and the spleen and lymph nodes may be enlarged. The characteristic histological lesion is multifocal, suppurative to pyogranulomatous inflammation of the skeletal muscle and connective tissues of the limbs, head and trunk. Cardiac muscle and smooth muscle of the stomach, intestine and urinary bladder may also be affected. Myositis involving the circumference of the esophagus may be a hallmark of DIM in ferrets. The diagnosis of DIM is based on the clinical presentation, lack of response to treatment, and the characteristic lesions in the skeletal muscle. *AHL*

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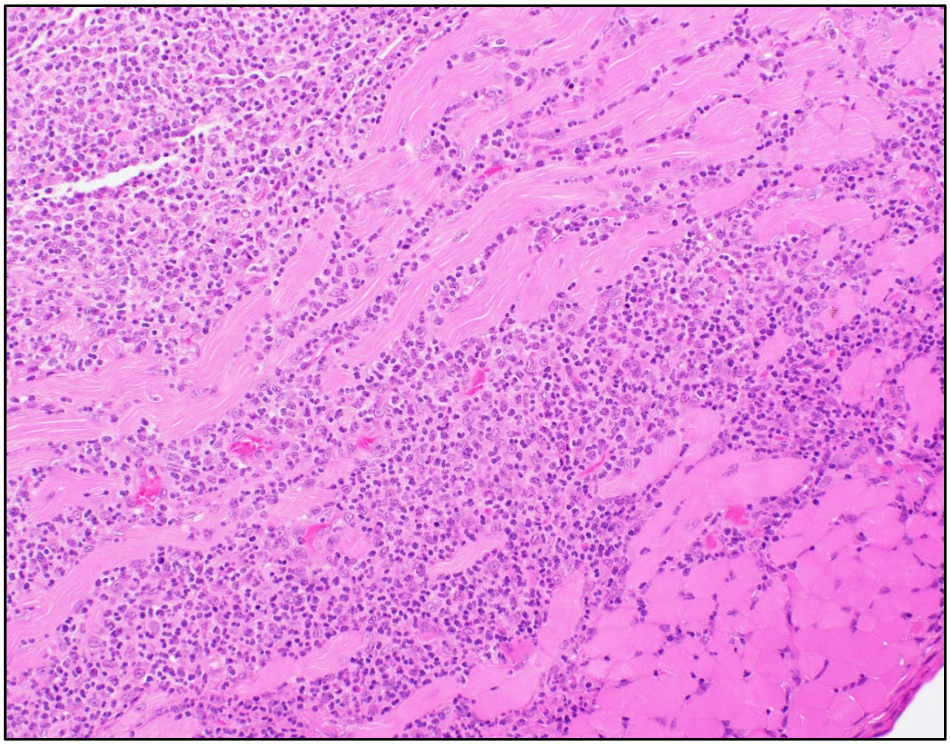

Figure 1. Suppurative to pyogranulomatous inflammation in the skeletal muscle. H&E stain.

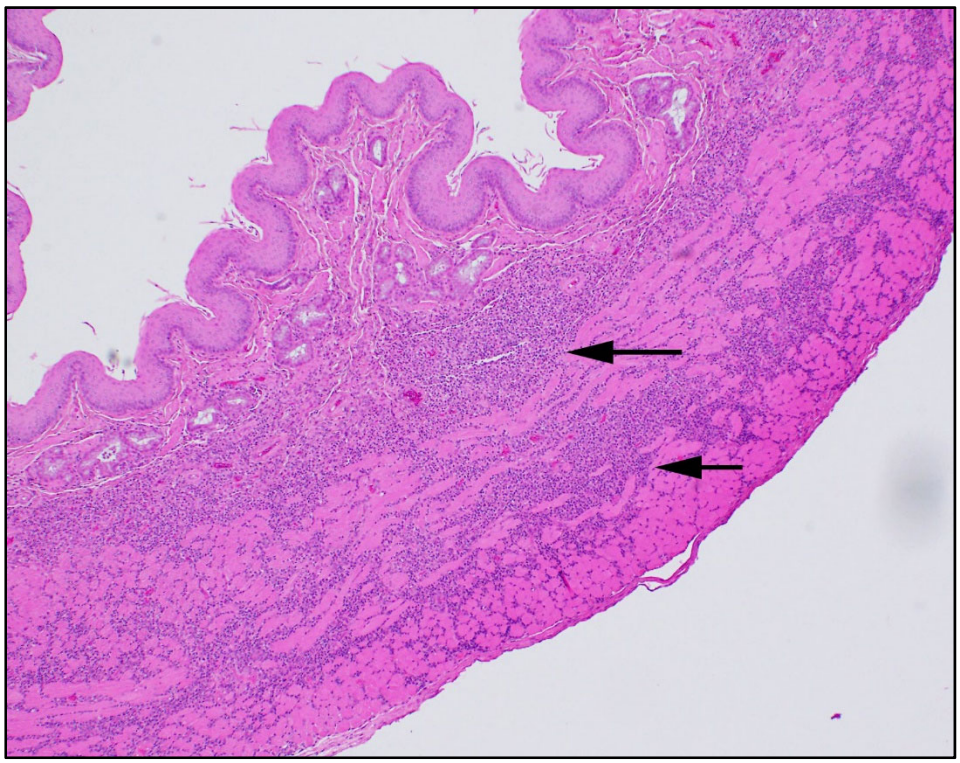

Figure 2. Inflammation involving the muscular wall of the esophagus (black arrows). H&E stain.

Baylisascaris-associated neural larval migrans in a group of juvenile Red-necked wallabies

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AHL Newsletter, 2021;25(2):20.

In early winter, a group of 8 to10-month-old juvenile Red-necked wallabies (*Macropus rufogriseus*) from a captive zoo population experienced an unusual disease event. Initially a single joey presented with sudden peracute death without any premonitory clinical abnormalities, followed shortly after by the onset of slow progressive neurological deterioration in a few juvenile cohorts. Neurological abnormalities included ataxia, proprioceptive deficits, opisthotonus, and *grand mal* seizures provoked by somatosensory or visual stimulation. An in-clinic postmortem on the first joey did not reveal any obvious gross abnormalities and a broad array of representative tissues were collected in formalin and submitted to the Animal Health Laboratory for histopathology.

Regionally within the white matter of the mesencephalon, brain stem, and cerebellum of this joey, there was prominent expansion of Virchow-Robbins spaces by dense aggregates of lymphocytes and macrophages up to ten cell layers thick (perivascular cuffing). Scattered throughout the adjacent neuropil were multiple irregular malacic foci that were filled with glial cells, Gitter cells, lymphocytes, gemistocytic astrocytes, and rare individual neutrophils. Given the predominantly mononuclear encephalitis, the concurrent onset of neurological symptoms in other individuals in this cohort, as well as a particular species susceptibility, acute toxoplasmosis was initially considered the most likely differential diagnosis. However, PCR testing of formalin-fixed paraffin embedded (FFPE) tissue scrolls, as well as IHC staining of affected brain segments, tested negative for *Toxoplasma gondii*.

Due to progressive neurological decline, and a concern to maintain quality of life, an additional affected joey was euthanized and both fresh and formalin-fixed brain along with other tissues were submitted for further investigation. Similar regionalized changes were present, this time within the brain stem, mesencephalon, and thalamus, however, both the perivascular and malacic inflammatory infiltrates also included small loose to moderately dense clusters of eosinophils. In addition, there were a few small oval to oblong linear pockets filled with dense aggregates of Gitter cells that resembled acute malacic tracts. Together with the eosinophilic inflammation, this was highly suspicious for aberrant metazoan nematode migration and indeed deeper sections of the malacic regions in the thalamic region revealed a few embedded characteristic ascarid nematode larvae and a diagnosis of neural larval migrans due to *Baylisascaris* sp. was made.

Further species confirmation was sought through a *Baylisascaris* sp. PCR test, targeting the mitochondrial Cytochrome-C oxidase subunit 2 gene (*COX2*). Both fresh frozen brain as well as FFPE scrolls from the thalamic block from the second joey and FFPE scrolls from affected brain in the first joey were tested; a positive result was obtained only from the FFPE scrolls from the second joey. Sequencing results of the PCR product were closely aligned to reported sequences for both *Baylisascaris procyonis* (raccoon roundworm) (305/306 base pairs, 99.67% identity) and *Baylisascaris columnaris* (skunk roundworm) (304/306 base pairs, 99.35% identity). Although this close match didn't allow for complete differentiation between the two species, the slightly higher homology and situational observation of multiple raccoon latrines on site suggest that raccoon roundworm remains the most likely etiology. Perhaps the curious nature of these young individuals and investigation of latrine sites placed them at a higher risk for exposure than the older cohorts in their mob.

B. procyonis is a well-known cause of neural larval migrans (cerebrospinal nemotodiasis), with infections having been recorded in many different species of birds and mammals. The eggs can remain infective in raccoon latrine sites for many years. The pathogenesis involves the emergence of larvae in the gastrointestinal tract which then migrate through the wall to the portal vasculature and liver, and then to the lungs where they enter systemic circulation and especially disseminate to the brain and eyes. Most cases are encapsulated in an eosinophilic granuloma, but in some cases, the nematodes migrate throughout the brain leaving behind necrotic tracts filled with debris and eosinophilic inflammation. In most cases the larvae are not visible in histological sections as the worms continue to migrate after the death of the animal. Other species of *Baylisascaris* could also cause similar lesions, although infection with these species is far less common than infection with raccoon roundworm: *B. melis* (badgers), *B. columnaris* (skunks), *B. laevis* (woodchuck), *B. schroederi* (pandas), *B. devosi* (fishers and martens), and *B. transfuga* (bears). Neural larval migrans from *Aelurostrongylus cantonensis* (rodent feces) is also a common differential.

Negative test results with the fresh brain samples was probably due to the multifocal nature of this infection, simply indicating that no worms were present within the pieces of sampled brain. This result, as well as the negative result obtained from FFPE scrolls from the first joey, probably also indicate minimal antigen shedding of the nematode during migration; therefore, best results with PCR testing would necessitate the physical presence of whole or fragments of nematodes in the sample. *AHL*

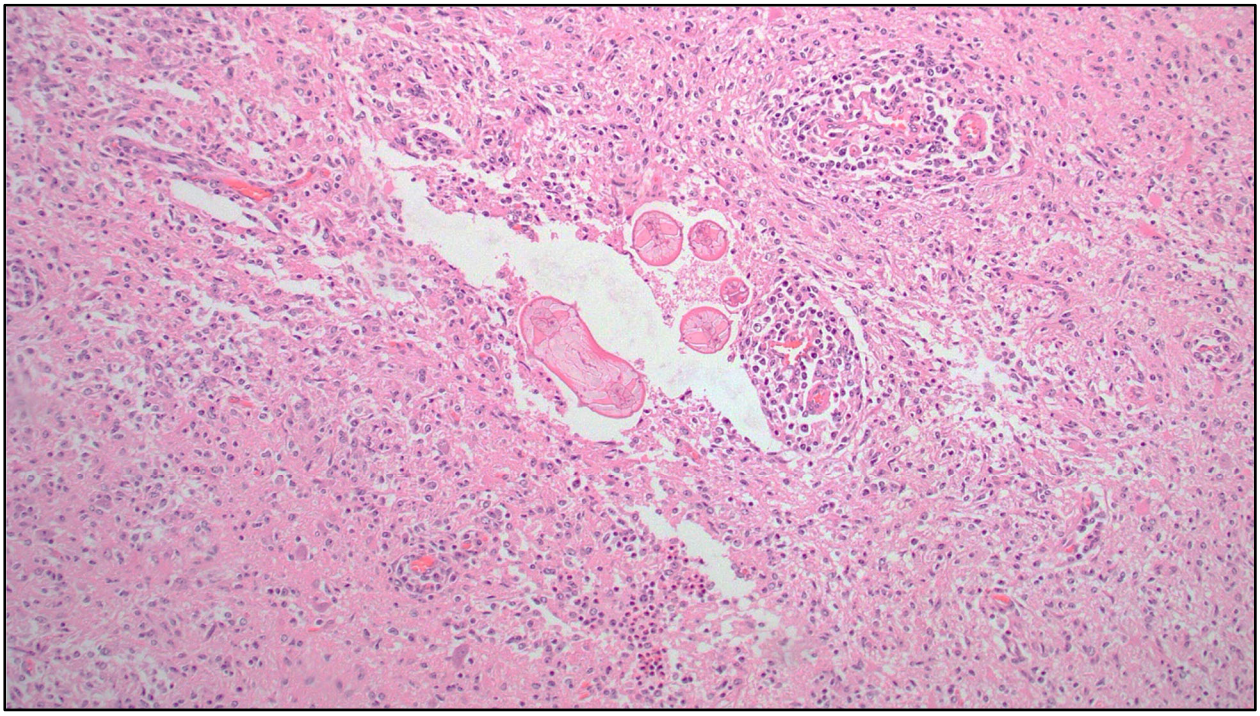

Figure 1. Brain (thalamus) with perivascular cuffing and scattered foci of malacia and gliosis with five adjacent embedded cross and tangential sections of nematode larvae consistent with neural larval migrans of an ascarid nematode. H&E stain.

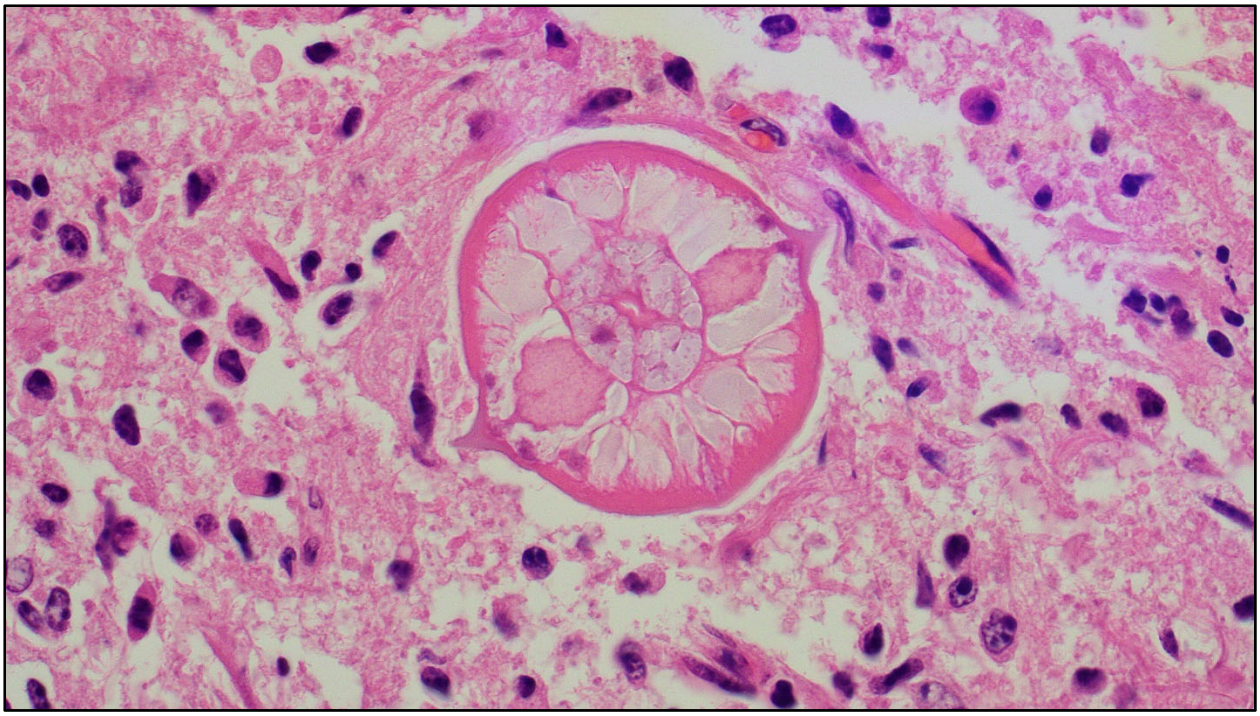

Figure 2. Larvae are ~ 30 to 50 µm wide with a 3-5 µm thick cuticle, prominent lateral chords and characteristic lateral alae, coelomyarian-polymyarian musculature, pseudocoelom, and an intestine lined by uninucleate columnar epithelial cells. Eosinophils are present within the adjacent neuropil. H&E stain.

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Lameness disorders in poultry

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Lameness in poultry can develop due to abnormalities in the skeletal, muscular, nervous or vascular systems. There can be subtle differences in the definitions, therefore, careful attention to detail is needed to determine the cause. The following are short descriptions of lameness disorders in poultry.

Developmental:

Angular bone deformity (valgus, varus): This is a long bone deformity. There is angulation of the distal tibiotarsal bone, deviation of the lower leg, and often bending of the proximal shaft of the tarsometatarsal bone. The tendon may be displaced. The legs curve together (valgus, feet lateral) or bow out (varus, feet medial).

Tibial rotation (twisted leg): Also a long bone deformity where the tibiotarsal bone is rotated through the long axis causing the leg to often extend laterally. The tendon is not displaced. This deformity is usually unilateral, can approach 90 degrees, and the cause is unknown.

Tibial dyschondroplasia (TD): Retained cartilage across part or entire width of the growth plate. Dyschondroplasia can occur in bones other than the tibia. Multiple contributing factors include: growth rate, Ca/P ratio in the diet, and electrolyte imbalance.

Spondylolisthesis (kinky back): Due to conformation and growth rate, the 4th thoracic vertebra is displaced, pinching the spinal cord resulting in posterior paresis and paralysis. Birds sit on hocks with feet slightly raised off the ground.

Splay legs: Occurs in birds less than 2 weeks of age. The legs twist laterally during development due to slippery surfaces. One or both legs are affected.

Slipped tendon (perosis): The gastrocnemius tendon is subluxated (slipped) due to hock deformation. The leg is malpositioned distal to the hock (usually laterally). Caused by nutrient deficiencies affecting growth plate development.

Dietary:

Rickets: Rapidly growing birds are susceptible to deficiencies or imbalances in Vitamin D, Ca or P. Malabsorption syndrome can also cause rickets. Birds are lame or, in subclinical situations, the flock can have poor performance, poor gait, and increased bone deformities.

Osteoporosis/Cage layer fatigue: Occurs in laying hens due to decreased mineralization of structural bone causing bones to be fragile and prone to fracture. Caused by high production (bone loss for egg shell formation), vitamin D/Ca/P deficiencies, age, housing, etc. Birds can be found paralyzed, alert and lying on their side. They can also be found dead with a shelled egg in the shell gland. The bones are fragile, brittle and may be fractured. The sternum may be deformed and the ribs infolded.

Infectious: Bacterial

Arthritis/Tenosynovitis: These conditions can develop after trauma, prolonged recumbency or secondary to a systemic infection. Acute lesions include redness, pain, heat and swelling. Joint exudate is opaque yellow, and yellow caseous deposits can develop within the joint. Chronic arthritis can lead to degeneration of articular cartilage, potentially affecting the underlying bone causing osteitis. Bacteria most often isolated include: *Staphylococcus aureus*, *Enterococcus cecorum*, and *E. coli*.

Bacterial chondronecrosis with osteomyelitis (BCO): Rapid growth results in excessive stress on growth plates of the proximal femur, proximal tibiotarsus, and the flexible thoracic vertebrae. This creates (osteochondrotic) clefts among the chondrocytes of growth plates. Circulating opportunistic bacteria colonize these clefts resulting in necrosis and granuloma formation within the growth plates at these 3 sites.

Septic osteomyelitis: This condition is similar to BCO and occurs in the same anatomic locations. It is secondary to bacteremia where circulating opportunistic bacteria settle out in the tight vasculature of the growth plates and also create areas of necrosis and granuloma formation. For both BCO and septic osteomyelitis, common bacteria isolates include: *Staphylococcus aureus*, *Enterococcus cecorum*, and *E. coli*.

Pododermatitis (Bumblefoot): These footpad lesions are due to trauma or poor litter condition and can lead to lameness. Secondary musculoskeletal lesions can include arthritis, osteomyelitis, and/or tenosynovitis. The AAAP has a footpad scoring guide available at this site: https://aaap.memberclicks.net/assets/documents/store/broiler%20paw%20scoring%20guide%20aaap%202015%20final.pdf

Infectious: Viral

Reovirus (viral arthritis): Reovirus infection can cause viral tenosynovitis primarily of the hock joint, and can sometimes result in rupture of the gastrocnemius tendon. Reovirus can also be involved in a malabsorption syndrome that could cause secondary osteodystrophies such as rickets and twisted legs.

Marek's Disease: This is a herpesvirus that can cause paralysis, lymphoid tumours and immunosuppression. It is ubiquitous in the environment and highly contagious. Infected birds develop a latent infection and continuously shed the virus. The virus causes inflammation in peripheral nerves such as the sciatic that can result in incoordination, progressive lameness or paralysis. A characteristic presentation is a bird with one leg stretched forward and the other back.

Infectious: Mycoplasma

Synovitis: *Mycoplasma synoviae* and *Mycoplasma meleagridis* cause infectious synovitis as well as respiratory disease. The joint exudate is translucent and gelatinous unlike the opaque exudate of bacterial infections.

Toxicity:

Ionophore toxicity: Severe myodegeneration of the adductor leg muscle results in reluctance to move and lameness. This is usually caused by feed mixing errors related to ionophore coccidiostats.

Other:

Femoral Head Necrosis (FHN): This condition is a postmortem description with multiple potential causes including: bacterial osteomyelitis, iatrogenic trauma, osteoporosis and rickets.

Spontaneous bone fracture: Fractures can be caused by rough handling, especially when catching for slaughter.

Please refer to the companion article published in the AHL March 2021 newsletter explaining how to examine and sample cases of lameness with accompanying photographs. *AHL*

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Spotlight on equine hemangiosarcoma

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AHL Newsletter 2021;25(2):25.

Hemangiosarcomas are uncommon neoplasms in horses. These malignant tumors are composed of neoplastic endothelial cells often lining variably-sized vascular spaces (**Fig. 1**). As a result, hemorrhage is a common clinical and histologic feature. Equine hemangiosarcomas may present as solitary neoplasms at a variety of anatomic sites, or as disseminated tumors involving multiple organs. In horses, hemangiosarcomas have been described in skin, ocular and periocular sites (limbus, conjunctiva, third eyelid, uvea), kidney, heart, brain, lung, and other internal organs.

Between May 2007 and May 2021, 9 cases of equine hemangiosarcoma were recorded in the Animal Health Laboratory database, including 4 postmortem cases and 5 biopsies. Solitary tumors occurred in 6 horses and were cutaneous (2), ocular (limbus or third eyelid - 2), or involved soft tissue (neck -1 or unknown site-1). Disseminated tumors in 3 horses involved 2 or more internal organs, including skeletal muscle and kidney (1), liver and spleen (1), and kidney, ovary, brain, lung, epaxial muscle, and vertebrae (1).

The age range for all horses with hemangiosarcoma was 3 months to 22 years (mean 10.6 years, median 11.5 years). Age was unknown for 1 horse in the series. Horses with solitary tumors tended to be younger (age range 3 months to 15 years, mean 7.25 years) compared to those with disseminated neoplasia (age range 13-22 years, mean 16.2 years). However the total number of animals in this series is too low to draw significant conclusions about age distribution of tumor subtypes in horses. Other studies have described a subset of hemangiosarcomas in young horses <3 years of age, including congenital tumors, and with primarily skin and limb involvement.

Clinical signs in horses with hemangiosarcoma reflect the location of the neoplasm, such as abdominal pain and weight loss for tumors in kidney or other abdominal organs. Hemorrhage into body cavities may occur in horses with tumors involving internal organs and may manifest as anemia. The clinical differential diagnoses for the 2 cutaneous tumors in this series were sarcoid and exuberant granulation tissue (proud flesh), both of which are more common equine cutaneous lesions; histopathology of lesion biopsies is required for differentiation. Exposure to sunlight is suggested in the pathogenesis of ocular hemangiosarcoma and in cutaneous tumors in non-pigmented skin.

For solitary tumors, radical surgical excision is the treatment of choice, with the addition of ancillary therapy in some cases. Some reports describe a favorable prognosis for cutaneous and ocular hemangiosarcomas that are completely excised. However, extensive studies are not available for prognosis of localized equine hemangiosarcomas due to the low incidence of the neoplasm in this species. Prognosis is poor for disseminated hemangiosarcomas in horses and other species.

Thank you to former Ontario Veterinary College Department of Pathobiology graduate students for their diagnostic contributions to these cases: Dr. Hibret Adissu, Dr. Fernanda Castillo, and Dr. Courtney Schott. *AHL*

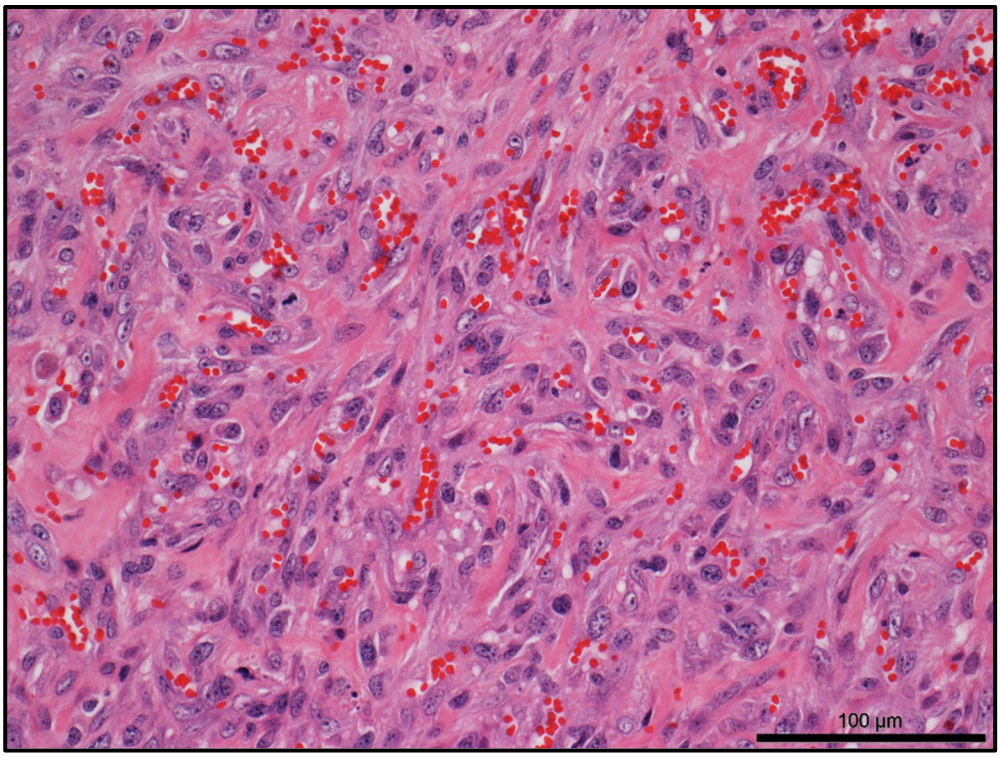

Figure 1. Cutaneous hemangiosarcoma in a 2-year-old horse. Plump neoplastic spindle (endothelial) cells forming multiple small, haphazardly arranged blood-filled spaces. H&E stain.

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Squamous cell carcinoma in the hoof of a horse

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AHL Newsletter 2021;25(2):26.

A 20-year-old Quarter Horse presented to the veterinarian with a protracted history of non-weight bearing lameness in the left hind limb. The hoof was unbalanced with more substantial overgrowth noted on the lateral aspect of the foot. Substantial pain with application of hoof testers was mitigated with local anesthesia. Radiographs revealed a space occupying lesion associated with regionally extensive lysis of the lateral aspect of the third phalanx (**Fig. 1**). Euthanasia was elected, and the affected limb was disarticulated at the fetlock so that the hoof could be submitted to the AHL for gross examination and histopathology.

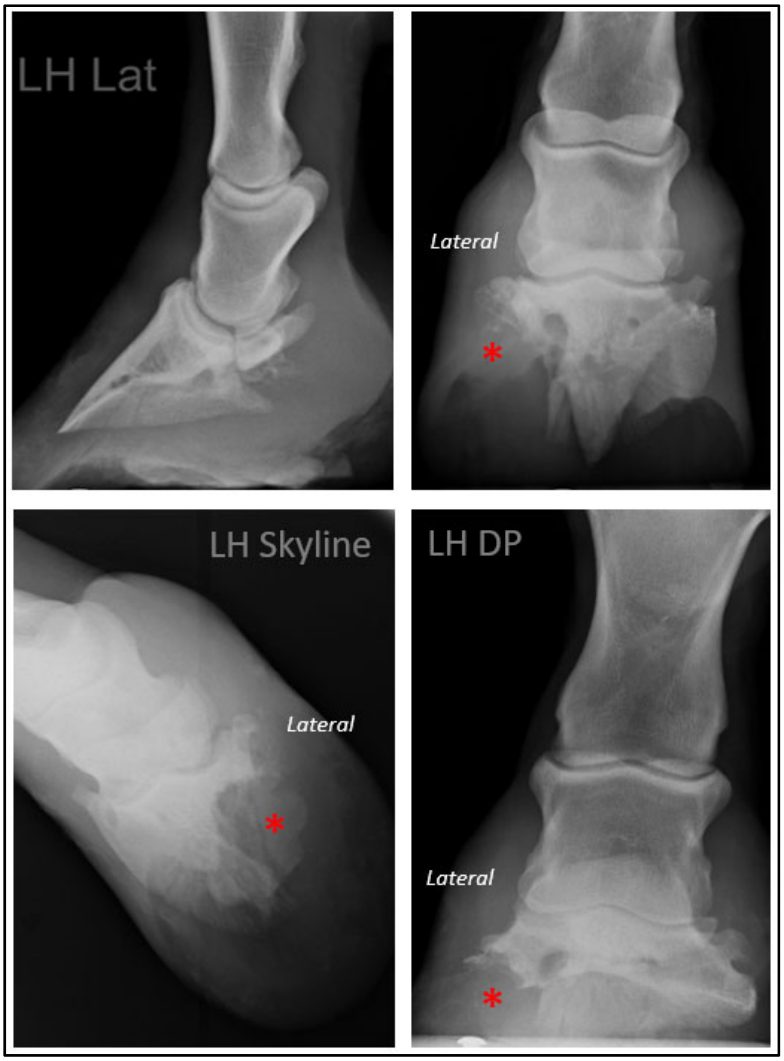

Figure 1. Radiographs of the left hind hoof, with asterisks (*) denoting a soft tissue opacity associated with regionally extensive lysis of the lateral aspect of P3.

Gross examination confirmed the presence of an ill-defined mass arising from the latero-plantar aspect of the hoof laminae/corium causing regionally extensive lysis and effacement of the distal phalanx. Parasagittal sectioning showed mild congestion surrounding the tip of P3, which was thinned and displaced dorsally by the irregularly thickened sole. Next, both halves of P3 were removed from the hoof, and dissection confirmed the presence of a mass arising from the hoof laminae/corium locally infiltrating and replacing the lateral and plantar aspects of P3. Grossly, the mass was comprised of slightly nodular, firm, tan-white tissue, and there were occasional short tan-white streaks (compatible with keratin) within the tissue adjacent to the plantar aspect of P3 (**Fig. 2**).

Histologic examination revealed squamous cell carcinoma, which occurs fairly commonly in the skin of horses, but reports of this tumor being identified in the equine hoof are sparse (1, 2). In this case, the distal phalanx was effaced by a mass of squamous epithelial cells arranged in variably thick trabeculae and islands supported by abundant collagenous stroma (desmoplasia) (**Fig. 3**). The neoplastic cells displayed disorderly maturation with frequent central keratinization, forming either a small central keratin pearl or a large central lake of keratin. Occasionally the thinner trabeculae were slightly reminiscent of hoof laminae. Overall, the neoplastic cells displayed up to threefold anisocytosis and anisokaryosis, frequent single cell death, patchy intracellular and intercellular edema, and infrequent mitotic figures.
AHL

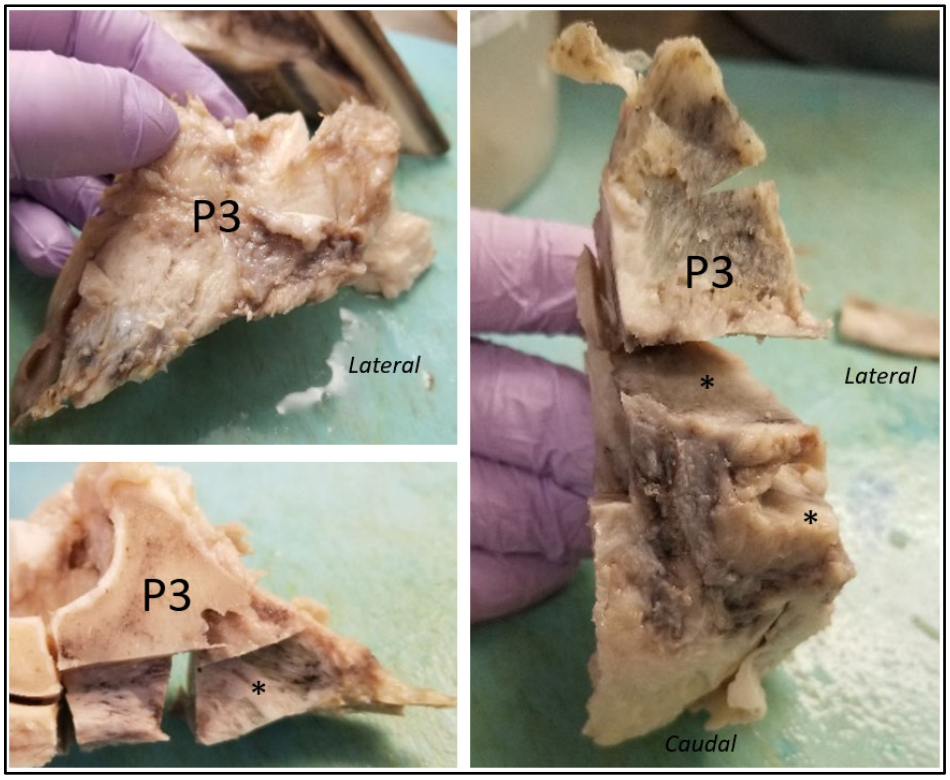

Figure 2. Gross images of the lateral half of P3, with asterisks (*) denoting an infiltrative mass effacing bone.

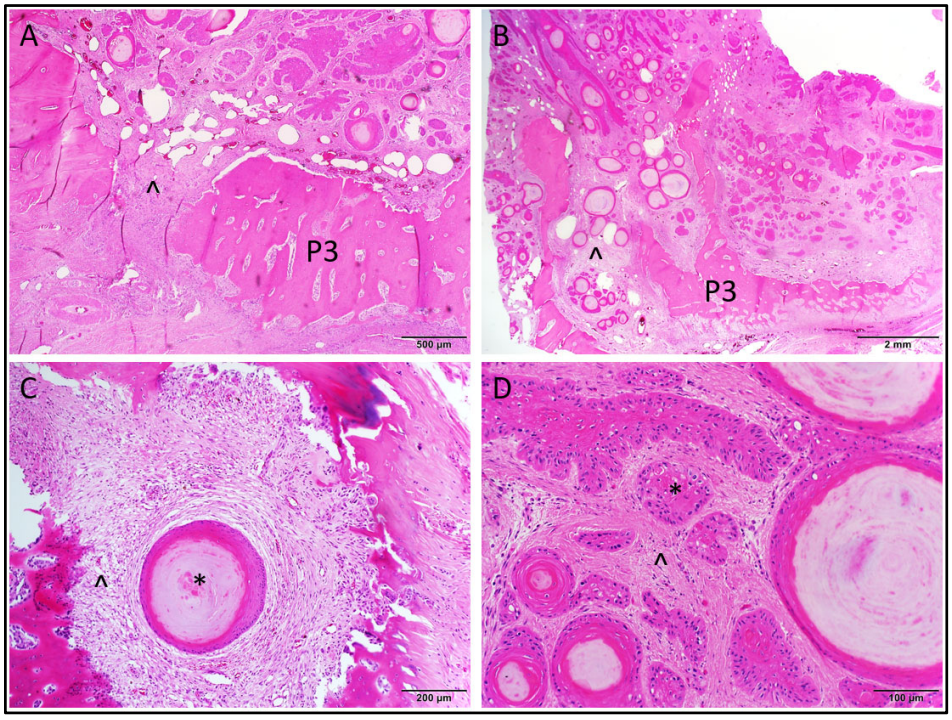

Figure 3. Histologic images of the infiltrative mass effacing the bone of the distal phalanx, with asterisks (*) denoting neoplastic epithelial cells and arrowhead (^) indicating the supporting fibrous stroma. H&E stain

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Acetaminophen intoxication in a dog

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A 5-year-old, female spayed Australian cattle dog developed acute vomiting and non-hemorrhagic brown diarrhea after spending time off-leash in a park. Radiographs were performed at the family veterinarian and granular mineralized material was observed in the stomach. The patient was referred to the Ontario Veterinary College Health Sciences Centre (OVC-HSC) emergency hospital for supportive care and further diagnostic testing. Upon presentation, the patient was estimated to be 5% dehydrated, had brown mucous membranes, bilateral conjunctival edema, and an increased respiratory rate (up to 50 breaths per minute, reference interval (RI) 20-34/min) and effort. Venous blood gas revealed mild respiratory alkalosis (pCO2 32.3 mmHg, RI 36-52 mmHg) and markedly elevated methemoglobin levels (25.6%, RI 0-2.2%) (1).

Given the timeline of clinical presentation, it was suspected that the patient was exposed to a toxin at the park. Acetaminophen intoxication was the primary differential diagnosis; therefore, appropriate treatment was initiated. Other possibilities included onion, garlic, or naphthalene intoxication (2).
At presentation to the OVC-HSC, the hematocrit was 0.48 L/L (RI 0.39 – 0.56L/L), but two days later there was a moderate normocytic normochromic anemia (hematocrit 0.23 L/L (RI 0.39 – 0.56L/L)) with marked poikilocytosis consisting of eccentrocytes, ghost cells and low numbers of schistocytes. Heinz bodies were present in about 80% of erythrocytes including on ghost cells (**Fig. 1**), indicating oxidative injury and intravascular hemolysis, respectively.

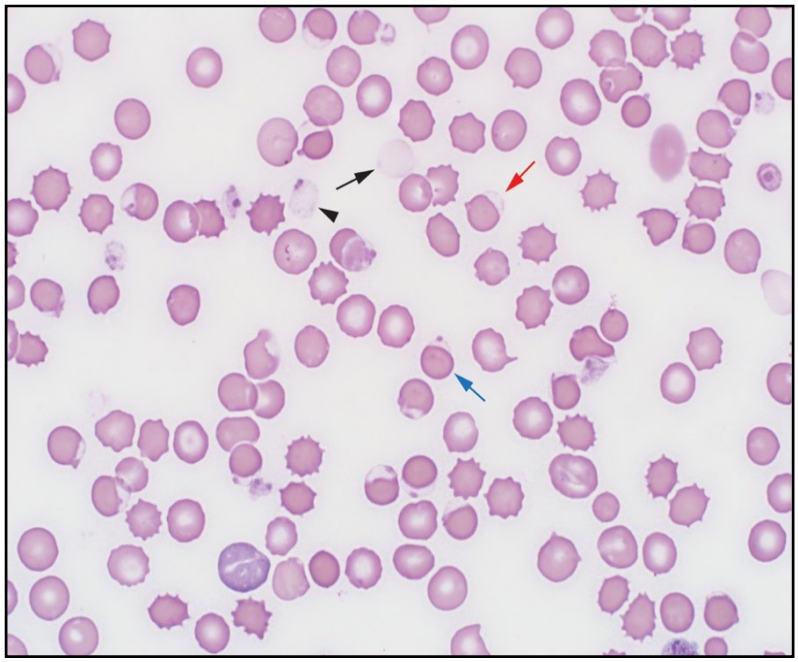

Figure 1. Blood smear with numerous eccentrocytes (red arrow), sometimes with associated Heinz bodies (blue arrow) and ghost cells (black arrow). The latter also occasionally contained Heinz bodies (black arrowhead). Wright's stain (1000x).

The serum biochemistry profile revealed hepatocellular injury, as indicated by severely increased ALT activity with a peak of > 10844 U/L (RI 19 – 107 U/L) on day 2 of hospitalization. On the third day, ALT decreased to 4503 U/L (RI 19 – 107 U/L)), and total bilirubin was 203 umol/L (RI 0-4 umol/L). The patient developed facial swelling, hemorrhagic and watery diarrhea, hemoglobinuria, continued hemolysis, and persistent methemoglobinemia. Given the poor prognosis, humane euthanasia was elected. On postmortem evaluation, there was icterus, subcutaneous edema, zonal hepatopathy with multifocal areas of pallor (compatible with centrilobular necrosis), bladder hemorrhage, kidney pigmentation, and myocardial hemorrhage. There was severe hepatic necrosis with marked bile stasis on histopathology. The kidneys exhibited mild tubular necrosis with hemoglobin casts and mineralization. Hemorrhage was observed in multiple organs and was considered consistent with coagulopathy secondary to liver failure.

Acetaminophen toxicity is dose-dependent, and suspicion of intoxication is based on the presence of methemoglobinemia, hemolytic anemia and acute hepatic failure. Acetaminophen is metabolized in the liver via three pathways which include glucuronidation, sulfation and cytochrome P450 oxidation (3). In cases of overdose, there is an increased amount of N-acetyl-p-benzoquinone (NAPQI), a product secondary to the P450 metabolic pathway, which leads to cellular membrane damage, hepatic necrosis and nephrotoxicity characterized by proximal tubular necrosis (3). Erythrocytes undergo oxidative damage leading to methemoglobinemia (2).

In the current case, acetaminophen intoxication was initially a presumptive diagnosis based on compatible clinical signs and postmortem findings, but was later confirmed by serum acetaminophen measurement. A liquid chromatography-mass spectrometry test was performed which confirmed the presence of acetaminophen at an estimated concentration of 296 ng/ml. In the literature, toxicosis by acetaminophen is associated with plasma concentration greater than 200µg/ml (3). Here, the dose detected did not likely reflect the peak concentration in the patient's serum since the sample was collected up to three days after hospitalization and acetaminophen has a relatively short elimination half-life (~1 hr). *AHL*

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Flow cytometry confirmation of canine histiocytic sarcoma

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A 10-year-old female spayed Miniature Schnauzer presented to the family veterinarian for evaluation of acute onset of partial anorexia and lethargy. The patient was found to be mildly pyrexic and was tender on cranial abdominal palpation. Blood work demonstrated a normal complete blood count, and moderately elevated liver values [AST: 351 U/L (RI 16-55 IU/L), ALP: 407 U/L (RI 5-131 IU/L) and bilirubin: 10.9 umol/L (RI 0.0-5.2 umol/L.)]. Thoracic and abdominal radiographs showed a cranial thoracic mass, possible pneumonia in the cranial lung lobes, trace pleural effusion, and a small liver.

The patient was referred to the Central Toronto Veterinary Referral Clinic, Oncology Service, for further assessment. Thoracic auscultation noted diminished breath sounds in the ventral lung fields. A CT scan of the thorax demonstrated the presence of two thoracic masses. One large (7.2 x 6.5 x 9.3 cm), cavitary soft tissue mass was located in the right hemithorax, suspected to be arising from the cranial mediastinum.

Another smaller soft tissue mass (2.9 x 2.3 x 1.2 cm) was within the caudodorsal aspect of the left caudal lung lobe. The CT scan also showed multiple mildly to moderately enlarged lymph nodes within the thorax (cranial, mediastinal, tracheobronchial, and right sternal), mild bilateral pleural effusion, and mild microhepatica.

The primary differential diagnoses were metastatic carcinoma, thymoma, lymphoma, and less likely, sarcoma. Ultrasound-guided fine needle aspirates of the mediastinal mass and samples of pleural fluid were sent to the Animal Health Laboratory, University of Guelph, for further testing. Cytology of the mediastinal mass revealed a pleomorphic population of atypical round cells with moderate to abundant pale blue cytoplasm, discrete cytoplasmic vacuolation, round to oval nuclei with coarsely reticular chromatin, and single to multiple indistinct nucleoli. Binucleated and multinucleated cells were numerous and mitotic figures were also identified (**Fig. 1**). The pleural effusion cytologic preparation was mildly cellular, but a few large hyperchromatic round cells were also noted. The cytologic findings from the mediastinal mass were most consistent with histiocytic sarcoma (HS), however, a poorly differentiated anaplastic carcinoma could not be ruled out. To reach a definitive diagnosis, immunophenotyping by flow cytometry was performed on aspirates from the same mediastinal mass. This method identified a population of large mononuclear cells highly positive for CD14, CD18, MHC II and partially positive for CD4. These results indicated a monocyte/histiocyte population consistent with an exfoliating histiocytic sarcoma.

Histiocytic sarcoma is an aggressive neoplasm of the innate immune system, most commonly of dendritic cell origin. It can be localized or disseminated (1). In this case, the patient was diagnosed with the disseminated form of disease which is more aggressive. Left untreated, disseminated HS progresses quickly and is rapidly fatal (2). Chemotherapy with Lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea - CCNU) is most effective, with a reported median survival time of 106 days (3). The owner accepted the recommended chemotherapeutic treatment, and prednisone was also started to help decrease inflammation and support the patient's appetite. However, during the first course of treatment, the patient experienced increased lethargy, difficulty breathing and subsequently died, 61 days after thoracic radiographs at rDVM identified the thoracic mass. *AHL*

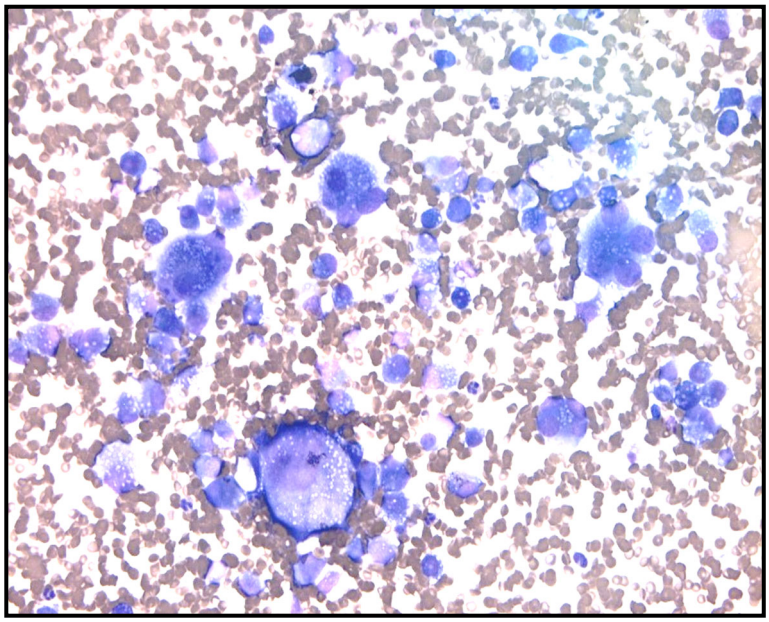

Figure 1. Aspirate of mediastinal mass showing a population of large neoplastic histiocytes with prominent criteria of malignancy, including numerous multinucleated cells. Wright's stain (600x).

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Disseminated mycobacteriosis in a Siberian cat (*Mycobacterium avium* subsp. *hominissuis*)

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A 3-year-old male neutered, indoor-only Siberian cat was presented to Alta Vista Animal Hospital on an emergency basis for dyspnea on August 28, 2020. Abdominal free fluid and enlarged lymph nodes were noted on examination. As the cat was maintained on a commercial raw food diet (rabbit meat), and on cyclosporine (Atopica) for possible atopy, a potential bacterial infection was suspected. Cytological evaluation of the aspirated enlarged lymph nodes showed neutrophilic inflammation. Empirical treatment with amoxicillin and clavulanic acid and pradofloxacin was initiated. Further testing was negative for FeLV, FIV and FIP, and no bacteria were isolated from routine aerobic and anaerobic cultures. PCR was negative for mycobacterium on the abdominal fluid and lymph node aspirates

The cat then represented to internal medicine on September 2nd, wherein aspirates of the lymph nodes demonstrated neutrophils and foamy macrophages with negatively-staining, intracytoplasmic rod-shaped bacteria, consistent with *Mycobacteria* spp. (**Fig. 1**). Treatment was initiated with clarithromycin, doxycycline and rifampicin.

On October 10th, the cat represented to internal medicine with ataxia, and a wide-eyed stare. Euthanasia was elected due to the progression of clinical signs (hyporexia, weight loss, icterus), and the body was submitted for postmortem examination.

On postmortem, multiple enlarged and coalescing abdominal lymph nodes were observed, and there were multiple adhesions throughout the abdomen. Histologically, there was severe granulomatous inflammation in multiple tissues, including: intestines, cecocolic and mesenteric lymph nodes, liver, bone marrow and spleen. Macrophages in all sites contained intracytoplasmic, acid-fast, rod-shaped bacilli consistent with *Mycobacterium* spp. (**Fig. 2**). Granulomatous inflammation in the section from the ileocecocolic junction was severe, effacing mucosal and muscular layers. No lesions were observed to explain the history of ataxia, though weakness secondary to systemic illness could not be ruled out.

Culture and PCR speciation performed by the Canadian Food Inspection Agency confirmed mycobacteriosis and speciation as *Mycobacterium avium*, subsp. *hominissuis*.

The species *M. avium* subsp. *hominissuis* can persist in the environment, making determination of the infection source difficult. Enteric localization with subsequent systemic dissemination suggests ingestion as a likely route of infection. Intestinal mycobacteriosis (*M. bovis*) resulting from a contaminated raw food diet has been reported in the United Kingdom (1). Disseminated mycobacteriosis with a largely enteric distribution was also observed in U.K. 'wild-type infections' (*M. avium* species identified in outdoor-only hunting cats), although cutaneous lesions were also observed in this population (2). Similar outbreaks have not been reported in North America.

The zoonotic transmission potential for this bacterial species in healthy individuals remains low, but a risk to immunocompromised populations remains. While one case does not represent an outbreak, there is value in raising awareness of systemic mycobacteriosis as a potential differential diagnosis for granulomatous enteritis and lymphadenomegaly in small animals. As intestinal mycobacteriosis can occur via ingestion, there is potential risk of transmission by raw food diets, consumption of small wildlife by domestic cats or other environmental contamination. In this case, it is suspected that previous cyclosporine use may have contributed to the establishment of infection. While the raw food diet was not confirmed to be the source of mycobacteria in this case (mycobacterial culture has been attempted with

results pending), it remains a strong potential source of infection in the absence of any likely environmental exposure. *AHL*

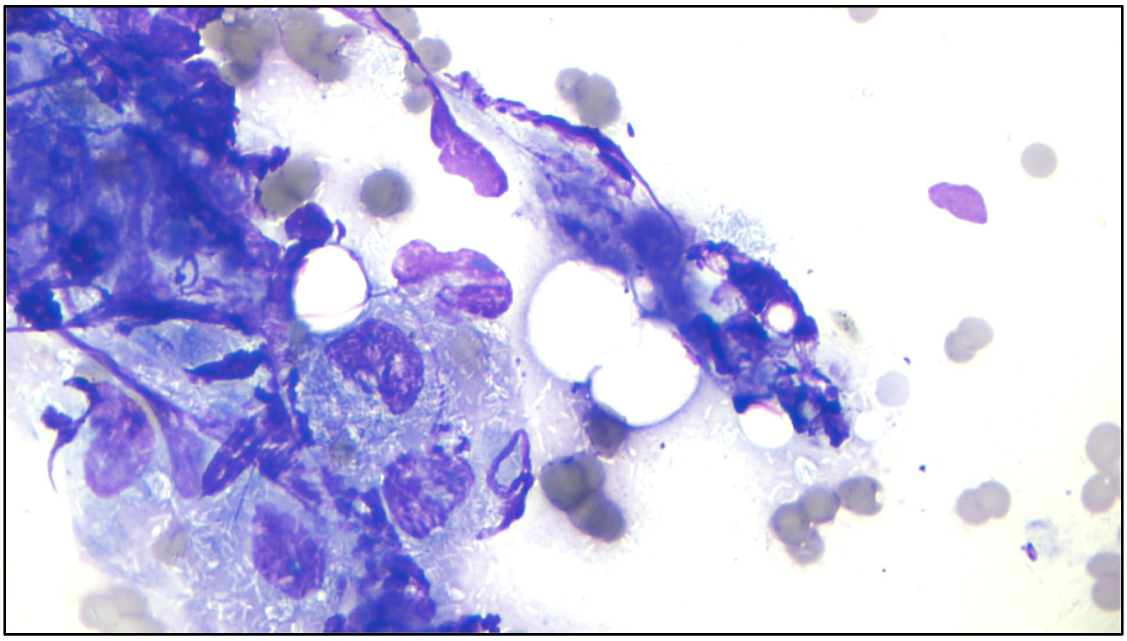

Figure 1. Cytology of foamy macrophages with negatively-staining, intracytoplasmic rod-shaped bacteria (consistent with mycobacteria spp.). Wright's stain (1000x).

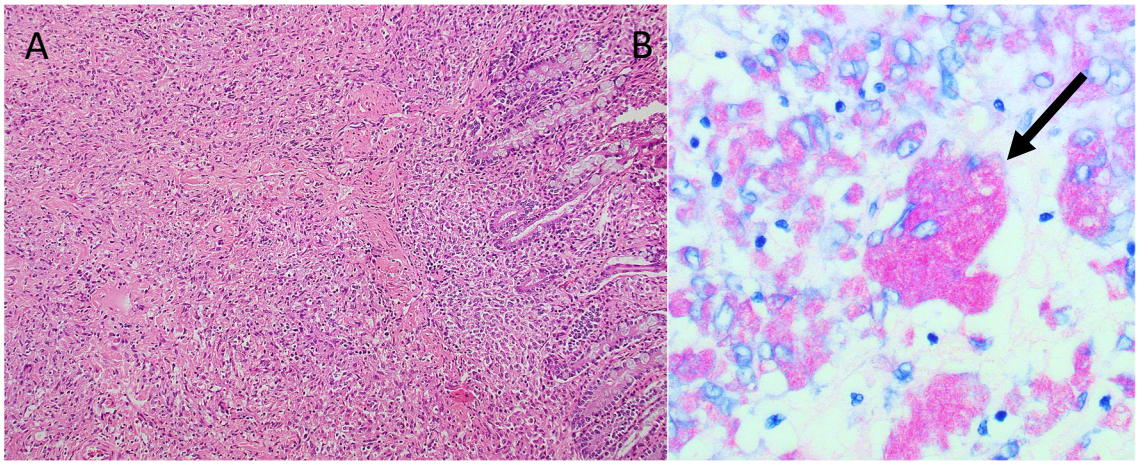

Figure 2. Transmural granulomatous inflammation in the colon associated with *Mycobacterium avium* subspecies *hominissuis*. 2A. Histologic section of colon illustrating numerous infiltrating macrophages effacing mucosal and muscularis layers. H&E stain. 2B. Multinucleated giant cell with acid-fast intracytoplasmic bacteria (arrow). Ziehl Neelsen stain.

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