Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

AHL Newsletter, Volume 26, Number 3 **September 2022**

In this issue:

AHL Newsletter
September, 2022 - Volume 26, Number 3 ISSN 1481-7179

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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. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

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Update from the Director

The view from the Director's office

It has been another busy summer at the AHL. Avian influenza testing has virtually disappeared from the commercial and backyard flocks, although positive signals continue to occur in wild birds. Check out the update on avian influenza in wild birds, kindly provided by the Canadian Wildlife Health Centre for this newsletter. Despite enhanced precautions, there are still COVID cases that cause challenges in staffing; however, our laboratory teams have done a stellar job in managing workload to accommodate vacations and to ensure cases meet the turnaround time expectations. It has been a great summer to date, and many of us are visiting friends and families that we haven't seen in person for a long time due to the pandemic.

The one certainty in any diagnostic veterinary laboratory is that there will always be new emerging and re-emerging diseases that confront us, requiring us to update our current diagnostic tests, or to develop new ones. For an example of the latter, we have an extensive article describing the cases of rabbit hemorrhagic disease in Ontario, a disease that is considered exotic to Canada (although there have been recent cases in Manitoba, Quebec and BC), and is on the CFIA list of immediately notifiable diseases. Nutritional diseases are featured in a number of articles in this newsletter, and there are also several case reports of uncommon disorders or unusual infections.

Despite the turnover in staff that all organizations are experiencing currently, the AHL is fortunate in being able to attract personnel with excellent credentials to fill these open positions. Please check out our Staff highlights section to view the cadre of young, enthusiastic professionals recently hired at the AHL. I'm sure you will agree that people are our greatest resource.

I hope you have been able to take some time off to relax and refresh before the busy fall months ahead.

Maria Spinato, Director

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

Getting the most from your veterinary diagnostic testing

Jim Fairles

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AHL Newsletter 2022;26(3):3.

The goal of ensuring that all diagnostic test results are meaningful and accurate is the responsibility of the veterinary practitioner, and ultimately, everyone involved in the diagnostic test process. Referral laboratories such as AHL have an extensive quality system in place to ensure that transmitted results are in accordance with quality standards. Quality programs should be considered for in-house diagnostic testing as well.

These are three main steps of the diagnostic test procedure that require consideration to eliminate errors and to ensure valid results:

Preanalytic phase
The preanalytic phase occurs before a diagnostic test is run. Sources of error during this phase include the following:

- mislabeling or not labeling a specimen tube
- allowing blood to clot before running a complete blood count
- accidentally introducing EDTA into a blood sample for chemistry analysis (by inserting a needle into an anticoagulant tube and then into a serum tube)
- improper preparation of samples for specialized testing

Analytic phase
The analytic phase is the actual sample analysis. Errors during this phase are associated with the diagnostic equipment or personnel performing manual tests, and can be caused by the following:

- expired reagents
- deteriorating equipment
- extrapolation of tests from one species to another
- presence of precipitate or contaminants in stains
- improper microscope maintenance
- human error (for example, incorrect interpretation of blood smears or fecal samples)
- failure to perform proper maintenance and quality control of in-house analyzers
- improper storage of urine dipsticks (allowing exposure to moisture or light)
- improper handling of urine dipsticks (dipping them vertically into a sample rather than keeping them horizontal)
- failure to account for factors like cleaning solutions that can influence results

Postanalytic phase
The postanalytic phase occurs after sample testing is completed. These errors involve data handling:

- incorrect data entry (transcription errors - entering the wrong numbers)
- assigning results to the wrong patient
- loss of data

Avoiding errors
Some recommendations to minimize errors include the following:

- use checklists to help avoid preventable errors
- use written procedures
- document personnel training
- ensure correct sample volumes and processing times
- document all errors
- perform and document instrument maintenance
- use properly stored, in-date reagents
- repeat tests when results are not compatible with a patient's clinical presentation
- back up all data

Quality results are everyone's responsibility! *AHL*

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5. Specimen Reception update – more on getting the most from your diagnostic laboratory. Jim Fairles. AHL Newsletter 2021;25(3):2 https://www.uoguelph.ca/ahl/specimen-reception-update-%E2%80%93-more-getting-most-your-diagnostic-laboratory
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OAHN Update – September 2022

Mike Deane, Tanya Rossi

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

This summer, the Ontario Animal Health Network has published many helpful and information-filled resources, as well as focusing on the 2022 Varroa Mite Campaign, equine veterinary webinars, and tick/Lyme awareness. In addition, we have held our quarterly species-specific network meetings and created the resultant veterinary and owner/producer reports which reflect what is being seen in veterinary practice, labs, and abattoirs throughout Ontario. To view the reports, go to OAHN.ca and navigate to the species you are interested in.

New Resources

- Infographic: Must Know Facts About Canine Distemper
- Veterinary Checklist: Dogs imported from Ukraine
- Tick Checklist for Dog Owners
- Monitoring and Diagnostics for Salmonella Dublin (must be registered and logged in to view this resource)
- An overview of the Anatomy, Physiology and Behaviour of Heat Stress in Poultry

OAHN Equine Veterinary Webinars

The OAHN Equine network has scheduled a series of equine medicine-focused webinars for Ontario equine veterinarians. Topics covered include: sustainable parasite control, *Borrelia burgdorferi* in the horse, vitamin E and selenium, pituitary pars intermedia dysfunction, and equine asthma. To access recordings of the two completed webinars, and to see which webinars are coming up in the future, please click here: https://www.oahn.ca/resources/oahn-equine-webinars-2022/.

New Reports

- Surveillance: Q1 Animal Health Laboratory data
- Bovine leukemia virus testing in Ontario
- Outbreaks of *Mannheimia hemolytica*
- Vet tip: Getting the most out of histology submissions
- Report a bovine disease to OAHN

- Avian influenza update: Poultry veterinary survey highlights – Q2 2022
- Disease discussion

- Disease discussion
- Laboratory diagnostic reports
- Ontario slaughter statistics
- CanSpot ASF surveillance update
- International disease topics of interest summary

- Sample submission request – Please!
- OAHN project: EHV-1 in postpartum mares
- OAHN Foal surveillance data for Q1
- OAHN Adult surveillance data for Q1
- Network member reports

- OAHN survey: CIRDC, heartworm, RHDV2
- Dog import ban
- Monkeypox unknowns
- Rabies: Ontario hot spot, baiting begins
- More!

Staff highlights

Dr. Amanda Mansz completed a BSc in Applied Health Sciences at the University of Waterloo in 2003, and graduated with a DVM from OVC in 2008. After a short time in small animal clinical practice, she returned to Guelph to pursue an Anatomic Pathology residency and attained a DVSc in 2014 and board certification by the American College of Veterinary Pathologists in 2018. Amanda has been working in the AHL's mammalian pathology service since 2018, and has recently accepted a permanent appointment.

Dr. Siobhan O'Sullivan has accepted a continuing appointment track position as an anatomic pathologist at the Animal Health Laboratory. Siobhan has a DVM and DVSc in anatomic pathology from the Ontario Veterinary College, and has been working as a contract pathologist for the AHL for the past 2 years.

Ms. Laura Austin has accepted the position of Technical Supervisor in the Histotechnology laboratory. She has a BSc (Honors Zoology) and an MSc (Biomedical Sciences) from the University of Guelph. Her MSc research investigated neuron regeneration in the leopard gecko brain.

Antimicrobial susceptibility testing part 4 – Laboratory results interpretation

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AHL Newsletter 2022;26(3):8.

AHL previously published information about antimicrobial susceptibility test methods, selection of bacterial organisms, and selection of antimicrobials for antimicrobial susceptibility testing (AST). In this fourth article of the series, laboratory results interpretation is discussed. The full document is available on the AHL website as Labnote 64: https://www.uoguelph.ca/ahl/ahl-labnote-64-antimicrobial-susceptibility-testing-ast

When it comes to interpretation of AST results, the Clinical and Laboratory Standards Institute (CLSI) provides a breakpoint for different drugs which is translated into an interpretative category (i.e., S, I, and R). As per CLSI: "breakpoint is minimal inhibitory concentration or zone diameter value used to categorize an organism as susceptible, intermediate or resistant." When available, the Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee recommends use of veterinary-specific clinical breakpoints because they are determined based on microbiological characteristics, pharmacokinetic and pharmacodynamics parameters, and/or clinical outcome (**Table 1**). However, veterinary-specific clinical breakpoints are not available for every drug/bacterial species/disease combination. At present, there are veterinary-specific guidelines available for some veterinary pathogens associated with urinary tract infection primarily in dogs and cats, respiratory infections primarily in cattle and swine, mastitis in cattle, metritis, abscesses, and wound, skin and soft tissue infections. In some cases, human clinical breakpoints are still used, or results are extrapolated from similar veterinary drug/bacterial species/disease combination (i.e., *M. haemolytica* respiratory breakpoints are used to predict susceptibility of *Bibersteinia trehalosi* in bovine respiratory samples).

This is what it means to get S, I, or R results, as per CLSI:

"**Susceptible (S)** – a category defined by a breakpoint that implies that isolates with an MIC at or below or a zone diameter at or above the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficiency."

"Intermediate (I) – a category defined by a breakpoint that includes isolates with an MIC or zone diameter within the intermediate range that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of drug can be used. This category also serves as a buffer zone to prevent the inherent variability of antimicrobial susceptibility testing methods from leading to erroneous categorization."

"Resistant (R) – a category defined by a breakpoint that implies that isolates with an MIC at or above or a zone diameter at or below the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent within normal dosage schedules and/or that specific microbial resistance mechanism are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in isolates with similar phenotypes." *AHL*

Table 1. List of antimicrobial agents that have CLSI-approved veterinary specific breakpoints.

Antimicrobial agent	Canine	Feline	Equine	Bovine	Bovine mastitis	Swine
Amikacin	X		X			
Gentamicin	X		X			
Spectinomycin				X		
Amoxicillin-clavulanate	X	X				
Ampicillin	X	X	X	X		X
Cefazolin	X		X			
Cefovecin	X	X				
Ceftiofur			X	X	X	X
Cephalexin	X					
Cephalothin	X					
Penicillin G			X	X		X
Penicillin-novobiocin					X	
Danofloxacin				X		
Difloxacin	X					
Enrofloxacin	X	X	X	X		X
Marbofloxacin	X	X				
Orbifloxacin	X	X				
Pradofloxacin	X	X				
Clindamycin	X	X				
Pirlimycin					X	
Gamithromycin				X		
Tildiprosin				X		X
Tilmicosin				X		X
Tulathromycin				X		X
Doxycycline	X		X			
Minocycline	X		X			
Tetracycline	X			X		X
Florfenicol				X		X
Tiamulin						X

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RUMINANTS

Eimeria macusaniensis (E-Mac) intestinal coccidiosis causing acute fatal neurologic disease in an alpaca

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AHL Newsletter 2022;26(3):10.

Approximately three weeks after giving birth to a healthy cria, a ten-year-old female alpaca was presented to the herd veterinarian for acute recumbency with neurological signs including nystagmus, lack of menace response and lack of pupillary light reflexes. The animal was euthanized and presented to the AHL for postmortem examination, which revealed thin body condition, scleral injection, marked pulmonary edema with congestion, multiple subacute to chronic renal infarcts occupying less than 20 to 30% of the kidneys, and ample red-brown liquid small intestinal contents with progressively formed feces in large intestine and rectum. Gross inspection of the brain did not reveal any parasites and the parenchyma did not fluoresce under UV light. Pertinent histologic findings included eosinophilic to necrotizing enteritis with coccidial organisms surrounded by a large clear parasitophorous vacuole among enterocytes (**Fig. 1A**), diffuse pulmonary congestion and edema with thrombi, moderate hepatic lipidosis, and multiple subacute to chronic renal infarcts. There were no discernable microscopic lesions in brain (e.g., no overt cortical laminar necrosis, parenchyma necrosis, or inflammation) to suggest polioencephalomalacia, viral or parasitic infection.

This herd was being managed on pasture with hay and small amounts of grain, and this alpaca was vaccinated against clostridial disease two months prior to the onset of clinical signs. With the constellation of clinical, gross and microscopic findings, it was determined that this animal's rapid clinical decline with progression to seizure was very likely incited by metabolic disturbance and circulatory shock stemming from coccidiosis. Toxemia stemming from loss of the intestinal mucosal barrier and/or proliferation of clostridia with enterotoxin production could not be fully excluded as an alternative possibility, but histology did not reveal enteric or brain lesions typical of clostridial enterotoxemia.

In llamas and alpacas, several *Eimeria* spp. are considered non-pathogenic, with shedding of large numbers of oocysts by asymptomatic animals; however, *Eimeria macusaniensis* (E-Mac) is a well-established cause of clinical disease in adult camelids, in which diarrhea may or may not be present, and sudden death is a common presentation. The exact pathogenesis of fatal coccidiosis has not been fully elucidated, but rapid clinical deterioration in the face of metabolic derangements (including hyperglycemia, hypoalbuminemia, ketonemia and hypothermia) and circulatory collapse have been described. Host immune responses (or immunocompromise associated with pregnancy) and environmental exposure play a role in transmission and disease severity; therefore, assessment of other alpacas on this farm for evidence of diarrhea/loss of body condition and review of environmental hygiene was recommended. Fecal examination of several herd-mates revealed the presence of E-Mac oocysts (**Fig. 1B, 1C**) along with non-pathogenic *Eimeria* spp. and a variable number of GIN eggs. E-Mac has a long pre-patent period, so it is important to note that animals can develop disease prior to detection of oocysts in feces. Moreover, the shedding of large heavy oocysts in low number means that multiple fecal examinations using a sensitive centrifugal flotation technique with high density sucrose solution (specific gravity of approximately 1.3), such as the Cornell-Wisconsin method, is preferred. *AHL*

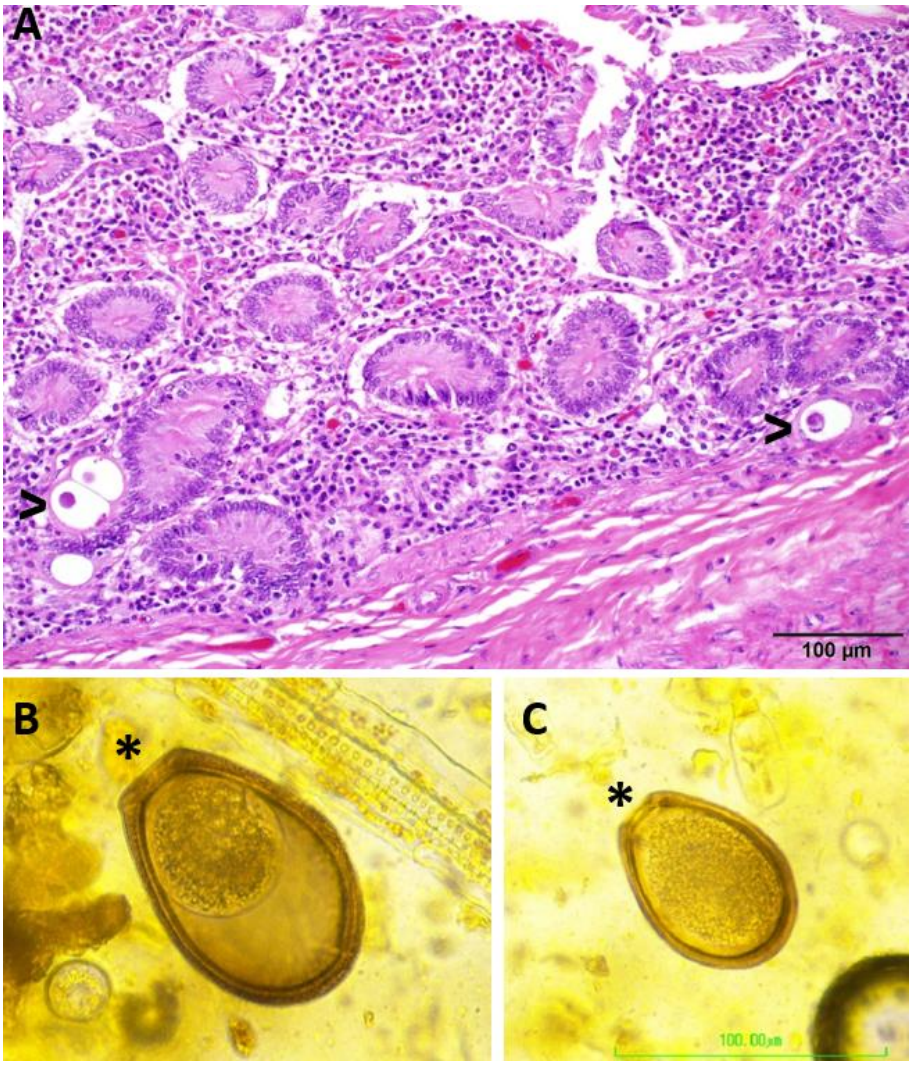

Figure 1. A. Histologic section (20x) of small intestinal mucosa containing eosinophilic inflammation and multiple coccidial organisms surrounded by a prominent parasitophorous vacuole within crypt epithelium (>). H&E stain. **B**, **C**. Fecal examination using the Cornell-Wisconsin method identified large (up to 110 um by 84 um) pyriform-shaped E-Mac oocysts that exhibit a thick (8.3 to 11.4 um) brown wall and with a micropyle and micropylar cap (*****).

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Bovine herpesvirus (BoHV-1) abortion in dairy cattle

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AHL Newsletter 2022;26(3):12.

Multiple aborted bovine fetuses were received from a dairy farm with a history of over 15 abortions occurring over a one month period. On postmortem examination, aborted fetuses were estimated to be approximately 6 months gestation, and there were no specific gross diagnostic lesions. Histopathology revealed multifocal acute hepatic necrosis in all fetuses (**Fig. 1**), and small foci of necrosis in the lung and adrenal glands of some fetuses. Fetal tissues were consistently positive for Bovine herpesvirus-1 (BoHV-1) by PCR with cycle threshold (Ct) values between 22 and 25. *Leptospira* sp. and *Neospora caninum* were not detected by PCR. The presence of typical microscopic lesions of infection combined with PCR detection of BoHV-1 in all fetuses confirmed this agent as the cause of abortions, despite this herd having a history of vaccination.

This was one of four total cases of confirmed BoHV-1 abortion submitted to the AHL in 2021-2022. Cases generally represent localized outbreaks of multiple aborted fetuses, often between 5-8 months of gestation. The characteristic histologic lesions of BoHV-1 infections are foci of necrosis in multiple organs, especially in the liver and often with intranuclear viral inclusion bodies in adjacent cells, as well as necrotizing vasculitis in the placenta. Gross lesions are not always evident, but can include white-tan foci of hepatic and/or pulmonary necrosis and renal hemorrhage.

In addition to abortion, BoHV-1 is also the causative agent of other recognized reproductive and respiratory production diseases, including infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/balanoposthitis. Viral latency and recrudescence following periods of stress are features of infection with BoHV-1, allowing infected but asymptomatic individuals to act as potential reservoirs of infection for other animals. The virus is transmitted between bovines by contact with respiratory, ocular, or vaginal discharges, and semen. Vaccination protocols are generally effective for prevention and control; however, vaccination of ill, stressed, immunocompromised animals, or missed or improperly timed doses, can result in breakthrough infections. *AHL*

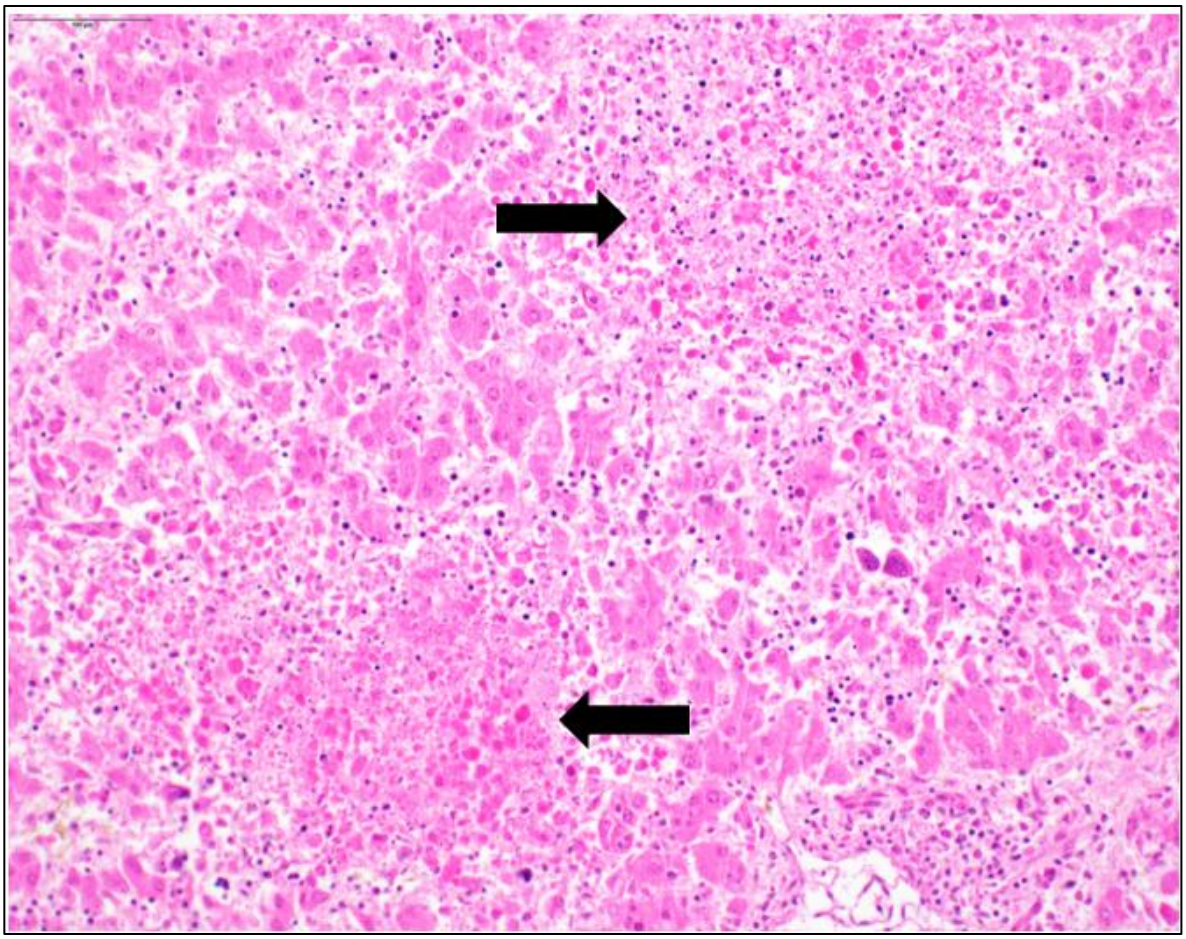

Figure 1. Bovine fetus, liver. Two foci of hepatic necrosis (arrows) with loss of hepatocytes, loss of differential staining, and accumulated cell debris. H&E stain.

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Phosphorus deficiency causing rickets in a group of nursery pigs

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AHL Newsletter 2022;26(3):14.

Approximately 4 days following arrival from the nursery, one 28 kg gilt was submitted to the AHL for necropsy to investigate suspected metabolic bone disease in a group of grower pigs. Several animals were exhibiting lameness and reluctance to move with minimal response to treatment with antibiotic and anti-inflammatory medications. Two on-farm necropsies revealed abnormal thoracic cages with ribs that were rubbery and bent prior to breaking. Necropsy at the AHL revealed similar rib changes including pliable rib bones and discrete nodular thickenings at costochondral junctions (rachitic lesions), and vertebral bodies and long bones that were either slightly pliable or brittle. On cut section, growth plates had an irregular or widened appearance typical of rickets. Most prominent in the humerii, there was obvious thinning and malalignment of the diaphyseal cortex which was brittle and easily broken with slight pressure. In addition, transverse sectioning of both scapulae revealed bilaterally symmetric step-like irregularities in the cortex with no mobility, consistent with chronic fractures. Dissection also revealed areas of hemorrhage in the muscles of the RH hip and femur and surrounding the RF elbow and shoulder. Histology confirmed failure of endochondral ossification which was most prominent in humeri and ribs, accompanied by fibrous osteodystrophy with osteopenia, medullary and periosteal fibrosis (**Fig. 1A**). In addition, a section of humeral growth plate displayed focally extensive tongues of retained hypertrophic cartilage (**Figs. 1B, 1C**). It was also evident that these changes led to instability and fractures in the scapulae and humeri, with resulting deposition of granulation tissue and periosteal new bone formation identified microscopically, along with adjacent areas of localized myonecrosis and hemorrhage.

Bone ash profiles of the second rib from two affected pigs revealed low bone ash (50% and 51%; normal reference range 58-62%), and reduced bone density (1.28 g/ml and 1.22 g/ml; normal reference range 1.4-1.5 g/ml), consistent with osteopenia. Bone ash levels of calcium and phosphorus were within the normal range, but this can occur in cases of metabolic bone disease where calcium and phosphorus deposits are present in relatively normal quantities within structurally abnormal bone. Overall, the constellation of clinical, gross and microscopic findings was highly characteristic of rickets, which is typically the result of inappropriate dietary levels of calcium, phosphorus, and/or vitamin D.

Further investigation uncovered a high likelihood of inappropriate phytase levels in the ration, thus phosphorus deficiency was presumed to be the inciting cause of metabolic bone disease in this group. Phytase supplementation of low-phosphorus (e.g., corn-soybean) rations is done to improve phosphorus digestibility and bone mineralization in growing pigs. In feedstuff derived from plants, phosphorus is primarily stored bound to phytate, and this form is mostly unavailable to pigs, with an overall digestibility of approximately 20 to 30%, therefore, the phytase enzyme acts to release phosphorus in a bioavailable form. Phytate can also form complexes with proteins and minerals leading to reduced nutrient absorption, so strategic application of phytase in swine diets is required to improve phosphorus digestibility, while also reducing the antinutritional effects. Use of phytase to improve utilization of phosphorus in feedstuffs

is advantageous, in that it also decreases the environmental impact of phosphorus excretion in swine waste and minimizes the use of expensive inorganic phosphorus. *AHL*

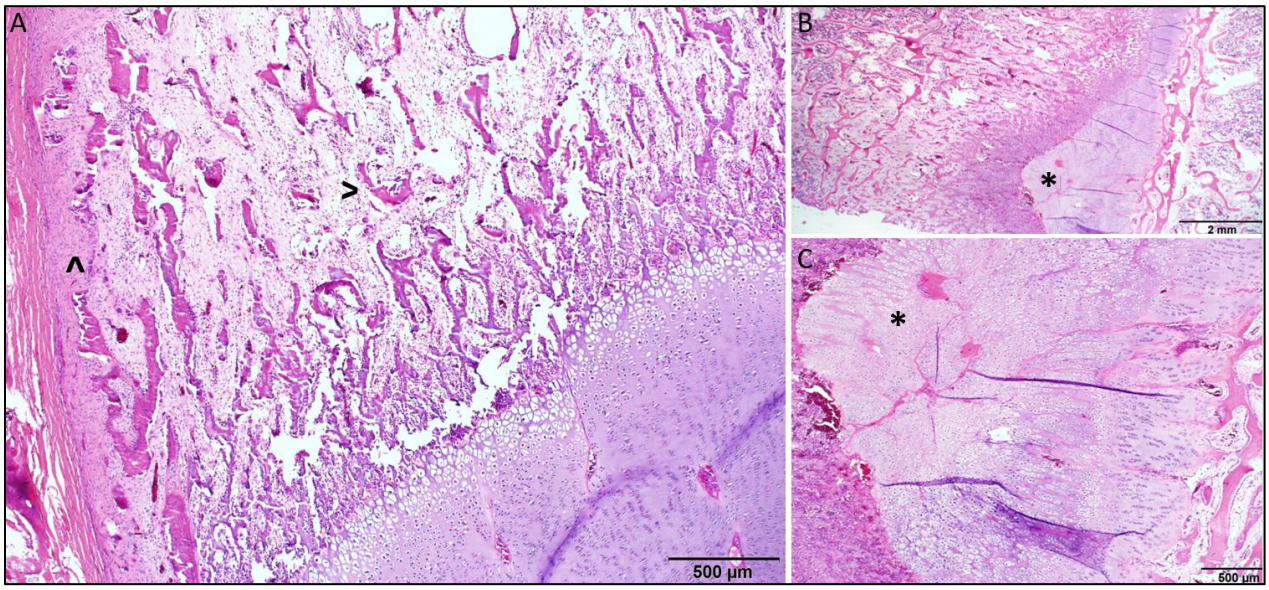

Figure 1. **A**. Microscopic section of humerus capturing growth plate and thin irregular trabecular (>) and cortical bone with failure of endochondral ossification and deposition of fibrous tissue (^) typical of fibrous osteodystrophy. **B, C**. Microscopic sections of humeral growth plate with focally extensive tongues of retained hypertrophic cartilage arranged in poorly organized columns that extend from the physis into the metaphysis (*****). H&E stain.

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Selenium deficiency in two Kunekune sows

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AHL Newsletter 2022;26(3):16.

Early in April 2022, two sows were presented for necropsy with a history of weakness progressing to an inability to stand or walk. Both sows were housed in the same pen with a feeding history of brewer mix and kitchen scraps. A mineral mix had recently been introduced. At postmortem examination, sows had a small volume of serous fluid present in the pericardial sac or abdomen. *Trichuris* spp. nematodes were observed in the intestinal lumens of both pigs. The underlying cause of the weakness was grossly inapparent.

In histopathology sections, there was marked diffuse polyphasic myodegeneration and necrosis in both the skeletal muscles (epaxial and postural muscles) (**Figs. 1A, 1B**) and heart (**Figs. 2A, 2B**). Mixed cellular infiltrates (predominantly histiocytic) were occasionally present in these areas, and were interpreted as cellular debridement and 'clean-up' of the necrotic myocytes. Other changes observed included focal areas of mild cellular necrosis in the kidney and liver of one pig which were interpreted as a response to terminal hypoxia or cellular apoptosis following stress.

Liver was submitted for vitamin E and selenium testing. In both sows, selenium was low to low normal (0.27 and 0.42 ug/g; normal reference interval of 0.4-1.2 ug/g); however, vitamin E was high normal in both (both 45 ug/g; normal reference interval of 15-50 ug/g). No other significant viral or bacterial pathogens were detected.

Inadequate selenium or vitamin E in the diet can result in the reduced capacity of cells to detoxify endogenously-produced peroxides (i.e., secondary to sudden environmental stress factors), leading to membrane damage due to free radical exposure. As part of this cellular damage, the resultant increased calcium influx overwhelms mitochondria, and causes hypercontraction, coagulation and degeneration of muscle fibres. These changes can be observed in the heart ("Mulberry heart disease") and/or skeletal muscle.

The presence of similar findings in two sows with the same housing and feeding history indicated the low selenium level as the most likely cause of the observed muscle findings, in the absence of any other potential underlying cause. The comparable lesions in each of these cases raises a question of whether or not there may be a susceptibility particular to this breed. However, there is no published literature addressing this possibility. In result, selenium deficiency should be considered a differential diagnosis for muscle weakness in pigs, and perhaps in Kunekune pigs in particular. The author would appreciate follow up with any reports of similar findings in this breed, if observed in the future. *AHL*

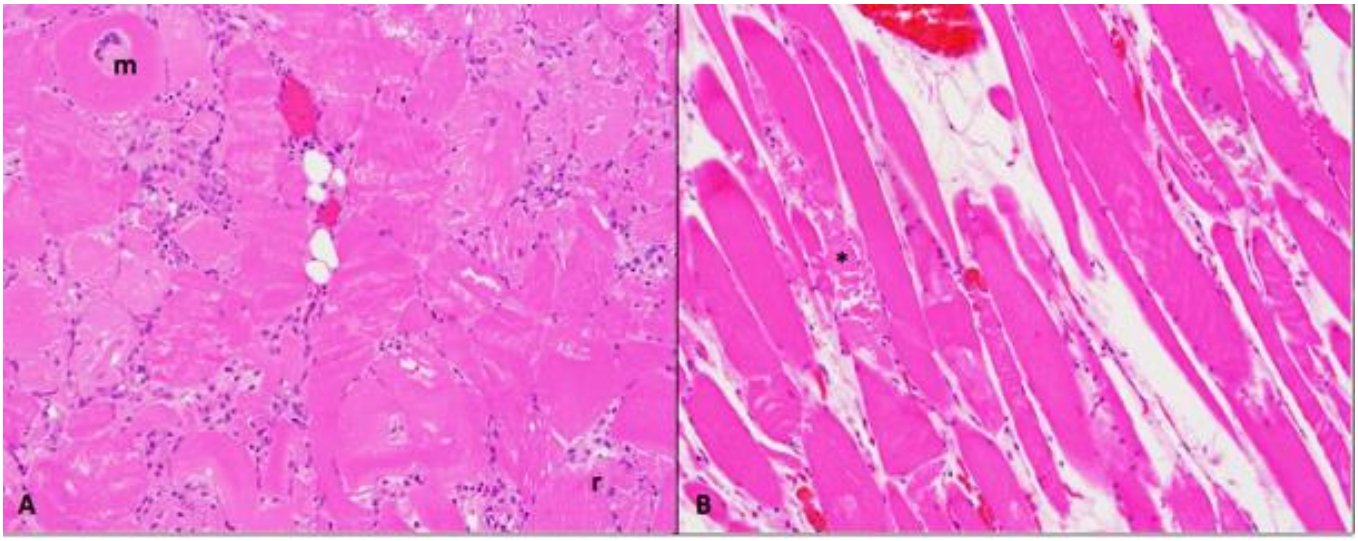

Figure 1. Polyphasic epaxial and postural muscle degeneration and necrosis in two Kunekune sows. **A**, **B.** Hypereosinophilia and fragmentation of myofibres (*), infiltration of macrophages (m), and early regeneration (r). 20x. H&E stain.

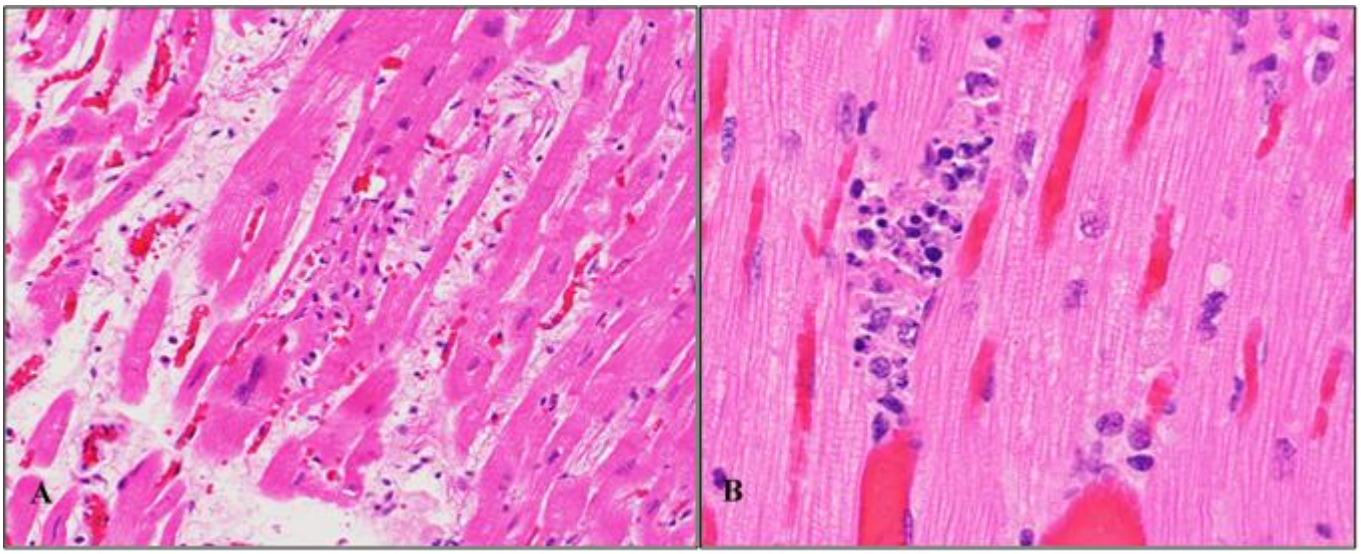

Figure 2. Myonecrosis in the hearts of two Kunekune sows. **A**-20x, **B**-60x. H&E stain.

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Rabbit hemorrhagic disease (RHD) identified in Ontario

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AHL Newsletter 2022;26(3):18.

AHL recently diagnosed rabbit hemorrhagic disease (RHD) in rabbits from 2 households. At the beginning of June, the initial household had 4 of 5 rabbits die within 6 days. The rabbits presented with lethargy and inappetence, proceeding to sudden death. The clinician performed postmortem examination on 3 of these rabbits. One rabbit had hydroureter and hydronephrosis of the right kidney, 1 rabbit (dwarf) had jaundice (**Fig. 1A**), and 1 rabbit had hemorrhagic fluid in the abdomen. Fixed tissues were submitted to AHL from the jaundiced rabbit. The same week, the 5th rabbit (Mini Rex) from this household was submitted to AHL from another clinic for postmortem examination. The history indicated the loss of the other rabbits and the problem list included anorexia (GI stasis), jaundice and kidney stones. On postmortem examination of the 5th rabbit, there was pulmonary congestion and mild abdominal hemorrhage consisting of multiple small blood clots and approximately 1mL of free hemorrhagic fluid.

On histopathology, the jaundiced dwarf and the Mini Rex both had acute hepatic necrosis ranging from single cell to multifocal coalescing areas of necrotic hepatocytes with primarily periportal distribution (**Fig. 1B**). The dwarf rabbit also had microvascular thrombi within renal glomeruli (**Fig. 1C**). Due to the number of acute deaths in 1 household and lesions of acute hepatic necrosis, tissues were directed to the National Centre for Foreign Animal Diseases in Winnipeg for rabbit hemorrhagic disease virus (RHDV) confirmatory testing. The tissues were positive for RHDV2 by PCR.

Due to widespread social media coverage, several subsequent rabbit postmortem submissions to the AHL requested testing for RHD; however, the next positive case of RHD was not detected until the end of June. This rabbit (dwarf) presented with seizures, became laterally recumbent, developed agonal breathing, and died within 2 hours of onset. Bloody fluid was noted at the nose and mouth. On postmortem examination at AHL, there was significant pulmonary hemorrhage. Histopathology identified alveolar hemorrhage and acute hepatic necrosis, similar to the rabbits from the initial household. Tissues were also directed to NCFAD for RHDV confirmatory testing, where they tested positive for RHDV2 by PCR.

In both households, the rabbits were noted as living indoors and there were multiple rabbits in each household.

Rabbit hemorrhagic disease virus (RHDV) is a calicivirus causing rabbit hemorrhagic disease (RHD). It was first reported in China in 1984 from imported rabbits. Since then, another strain has evolved and the nomenclature is being updated (**Table 1**). In general, the virus primarily affects European rabbits (*Oryctolagus cuniculus*), and is now endemic in multiple geographic areas where European rabbits also exist in the wild, including Europe, Asia, Africa, Australia, and New Zealand. The virus is highly contagious, has a short incubation period, and is rapidly lethal. The classical RHDV results in death of

adult domestic and wild rabbits within 12 – 35 hours after onset of fever (>40°C). The virus targets the lung, liver and spleen, causing B and T lymphocyte depletion in the latter two organs that subsequently impairs the immune response. Lesions primarily include acute necrotizing hepatitis, but hemorrhages can also be identified in lungs, heart and kidney due to DIC which is the usual cause of death.

Routes of transmission are through oral, nasal, conjunctival and parenteral routes (blood-feeding insects) as well as mechanical vectors. Transmission can be by direct contact (urine, feces, respiratory secretions from infected animals), fomites (contaminated food, bedding, water, clothing, cages), or vector-borne (mammals, birds, insects such as flies).

There are multiple forms of RHD described (*most common):
Peracute*: No clinical signs. Sudden death.
Acute*: Anorexia, depression, conjunctival congestion, neurologic symptoms (opisthotonos, ataxia, paddling, paralysis), respiratory signs (dyspnea, cyanosis, foamy and bloody nasal discharge), lacrimation, ocular hemorrhages, and epistaxis.
Subacute: Similar presentation to acute, but milder symptoms and most rabbits survive and develop protective antibodies.
Chronic: Can be seen in an outbreak. A few rabbits may have severe generalized jaundice, anorexia and lethargy. They tend to die in 1-2 weeks, but may survive and seroconvert. Surviving rabbits can continue to shed virus for up to a month after recovery.
NOTE: Rabbits less than 2 months of age usually have subclinical infection but rarely die. If infected, young rabbits develop immunity to related strains.

Since caliciviruses are non-enveloped, they are hardy and stable in the environment. Virus can be viable on carcasses for prolonged periods (months). Control relies on biosecurity measures such as surveillance, sanitation, disinfection, and quarantine, as well as vaccination. There is no specific treatment for RHD other than supportive care.

RHD has been previously identified in Canada: (RHDV (single rabbit): Winnipeg in 2012 (RHDV, single rabbit); Quebec in 2016 (RHDV2 outbreaks), Vancouver Island in 2018, and southern mainland of BC in 2019. This is the first identification of RHD in Ontario. *AHL*

Table 1. Rabbit hemorrhagic disease virus classification.

Classification: NOTE: RHDV and RHDV2 belong to different serotypes – there is little to no cross protection.	**Current Classification:** Family: *Caliciviridae* Genus: *Lagovirus*	**Species affected**
RHDV (classical) – identified 1984 - incubation 1-3 days - die 48-72 hours post infection - morbidity rate 30-100% - mortality rate 40-100%	- Subtype RHDVa - genotype GI.1 (new nomenclature 2017)	Adult European rabbits (*Oryctolagus cuniculus*)
RHDV2 or RHDVb – identified ~2010 - has replaced classical RHDV in many geographical areas - incubation 3-5 days - clinical signs develop in 3-9 days - mortality rate 5-70%	- genotype GI.2 (new nomenclature 2017)	Juvenile and adult European rabbits (*Oryctolagus cuniculus*) and hares (*Lepus* spp.)

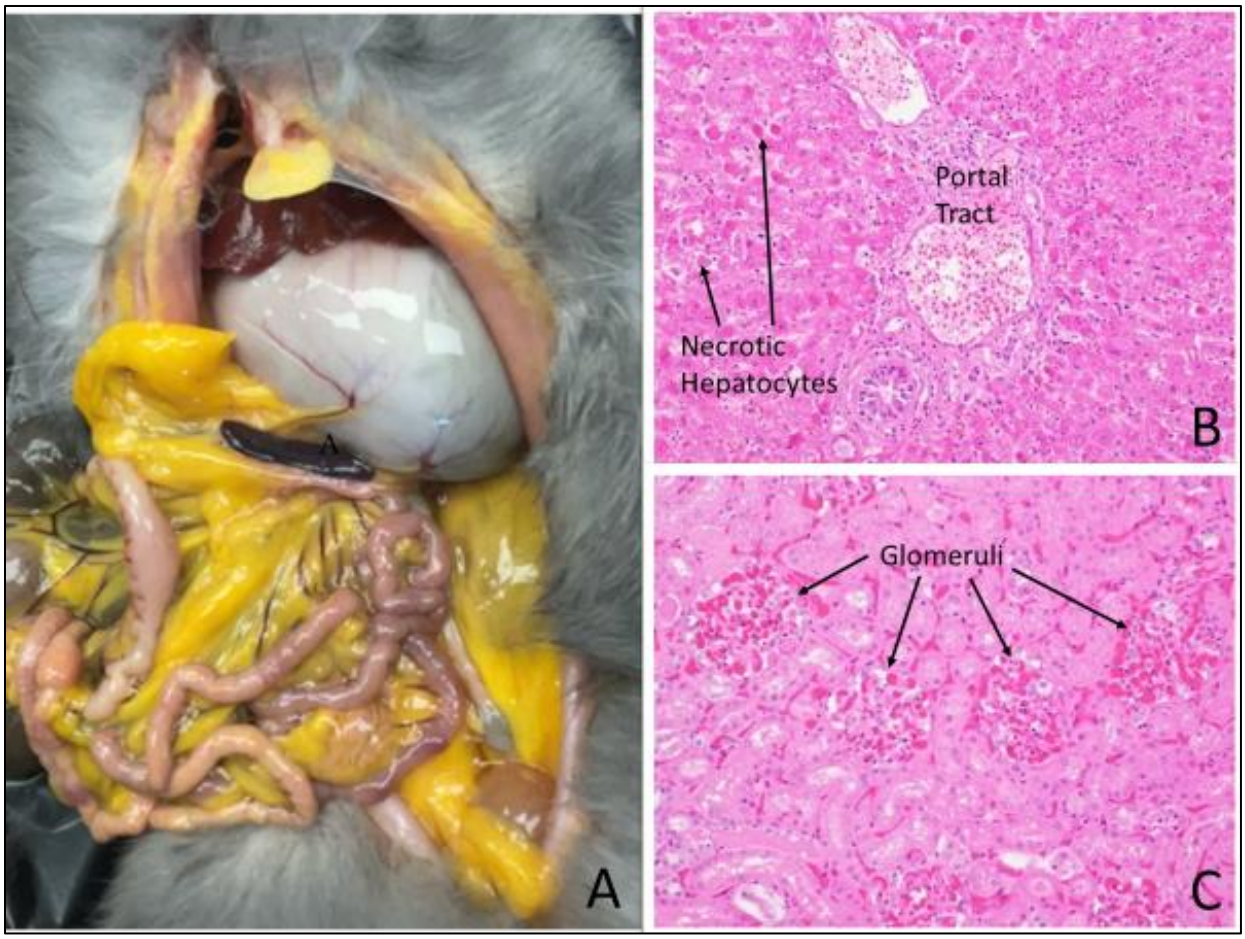

Figure 1. Postmortem changes and histologic lesions of RHD. (Photo A courtesy of Dr. Jamie McGill Worsley; Photos B and C by Dr. Emily Martin)
A. Jaundiced dwarf rabbit from first household.
B. Acute hepatic necrosis. H&E stain.
C. Microvascular thrombi in renal glomeruli. H&E stain.

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Update on avian influenza in wild birds

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AHL Newsletter 2022;26(3):20

The Canadian Wildlife Health Centre (CWHC) continues to diagnose cases of highly pathogenic avian influenza virus (HPAIV) in wild birds from Ontario. The majority of positive cases over the

summer have been from Northwestern Ontario, with rare cases in Southern Ontario. These positives in the Northern part of the province, along with the positive cases detected in Nunavut, are concerning as they indicates the virus is still circulating in certain wild bird populations. It is expected that as these birds begin to congregate and migrate south for the winter, case numbers will begin to increase in Southern Ontario again.

Continue to monitor the CWHC HPAIV dashboard http://www.cwhc-rcsf.ca/avian_influenza.php and the CFIA HPAIV dashboard https://cfia-ncr.maps.arcgis.com/apps/dashboards/89c779e98cdf492c899df23e1c38fdbc to obtain the latest information on the spread of HPAIV in Ontario. *CWHC*

Microfilaria in a panther chameleon

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AHL Newsletter 2022;26(3):21.

Direct smears of material obtained from scrapings of hyperkeratotic lesions on the lips and commissures of the mouth in a panther chameleon were examined cytologically. No additional clinical information was provided.

The slides contained mildly hemorrhagic backgrounds with clusters of both parabasal and superficial keratinized squamous epithelial cells intermixed with moderate numbers of lytic heterophils. Free heterophil granules were present within the slide backgrounds, along with numerous pleiomorphic extracellular bacteria, most consistent with resident flora. Also found were frequent extracellular microfilaria which exhibited both blunted and tapered ends, and a small clear sheath over the entire organism. Internal structures appeared coarsely granular in nature. These microfilaria were interpreted to most likely represent *Foleyella* spp., due to the presence of the loose sheath which completely enclosed the microfilaria. Definitive identification would require evaluation of an adult worm.

Filarial nematodes of various genera can affect reptiles. *Foleyella furcata* and *Foleyella brevicauda* are commonly reported in chameleons from Madagascar. In affected chameleons, adult worms are found in subcutaneous tissues. Following fertilization, the female worm releases large numbers of microfilaria into peripheral blood. Blood sucking arthropods such as *Culex* and *Aedes* mosquitoes serve as intermediate hosts, and transmit the infective larval stages to other reptiles. Thus, microfilaria can be found in peripheral blood, and adult worms are located within subcutaneous tissues and body cavities of affected animals. Most infections by *Foleyella* spp. are asymptomatic; however, heavy infestations and the anatomic location of the adult worms could produce pathologic changes such as thrombosis, edema and necrosis, with associated clinical signs. Immunosuppression or concurrent disease could possibly worsen the clinical signs and lesions.

It was unclear if the microfilaria identified in this submission were simply an incidental finding, an indicator of underlying poor health due to disease, husbandry, or nutritional issues, or if this organism contributed directly to this patient's clinically identified lesions. Ideally, a peripheral blood smear would have been assessed to ascertain the overall burden of microfilaria, and the hyperkeratotic lesions would have been evaluated histologically. Unfortunately no follow up testing was undertaken. *AHL*

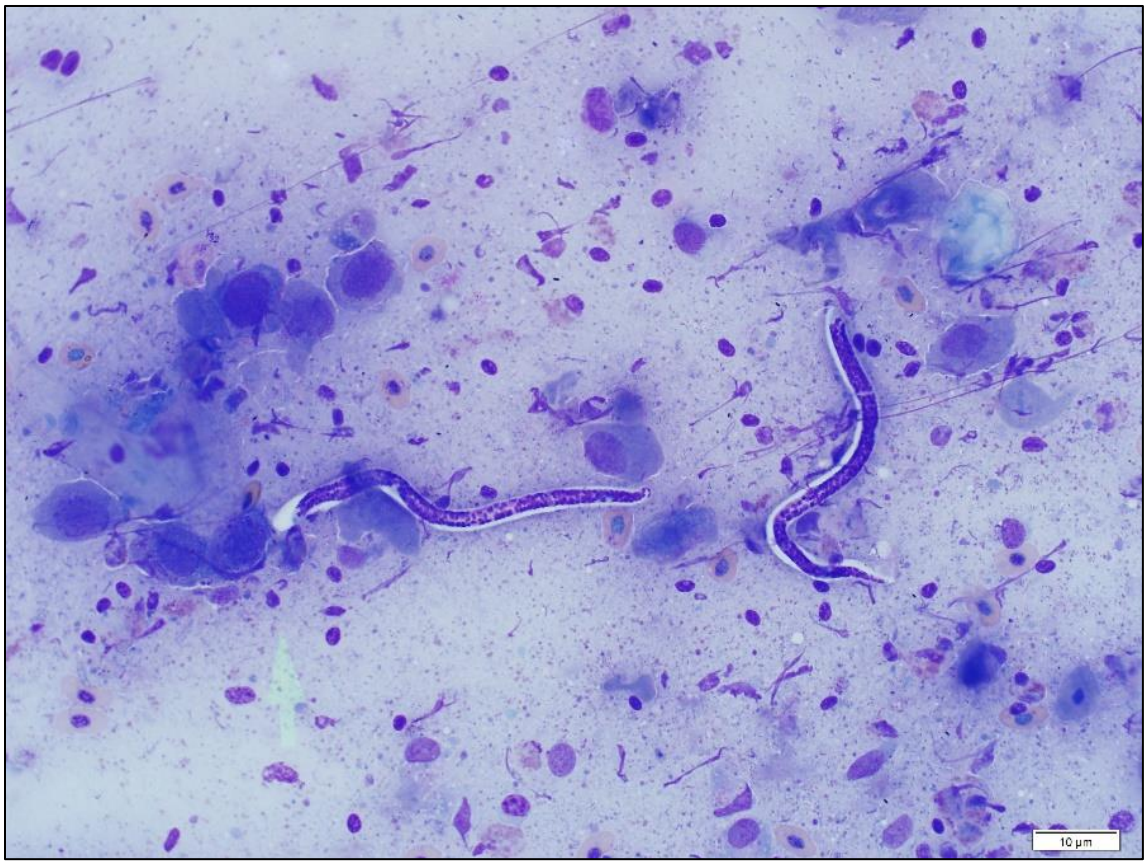

Figure 1. Microfilaria on a background of individual epithelial cells aspirated from a hyperkeratotic lesion on the commissure of the mouth. Wright's stain. (Image courtesy of Dr. Felipe Reggeti.)

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HORSES

Neonatal foal death due to a congenital defect

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AHL Newsletter 2022;26(3):23.

A full-term foal who survived only minutes after a difficult foaling was submitted to AHL for postmortem evaluation. Opening the abdominal and thoracic cavity revealed the entire liver, large colon, cecum, and ileum/distal jejunum were displaced into the thoracic cavity. The pelvic flexure of the large colon was located in the thoracic inlet **(Fig. 1)**. The lungs were displaced and compressed into the cranial dorsal thoracic cavity. The heart was also displaced slightly cranially, but the pericardium remained intact. It was determined that the abdominal organs shifted into the thoracic cavity through a 20 cm x 15 cm oval-shaped opening in the diaphragm, which extended from the sternal body wall to the ventral aspect of the esophageal/caval hiatus. The opening was rimmed by a smooth fibrous surface **(Fig. 1**).

In horses, congenital diaphragmatic hernias are a rare developmental lesion associated with stillbirth, intermittent bowel obstruction, or occasionally, newborn colic. In this case, the cause of the acute death shortly after birth can be attributed to the inability to inflate lungs due to massive compression by relocated abdominal organs and associated circulatory disturbances. The diaphragmatic rent was considered congenital due the smooth fibrous contour of the edge of the opening (no tearing or hemorrhage), and not a result of trauma from the dystocia described in the history. *AHL*

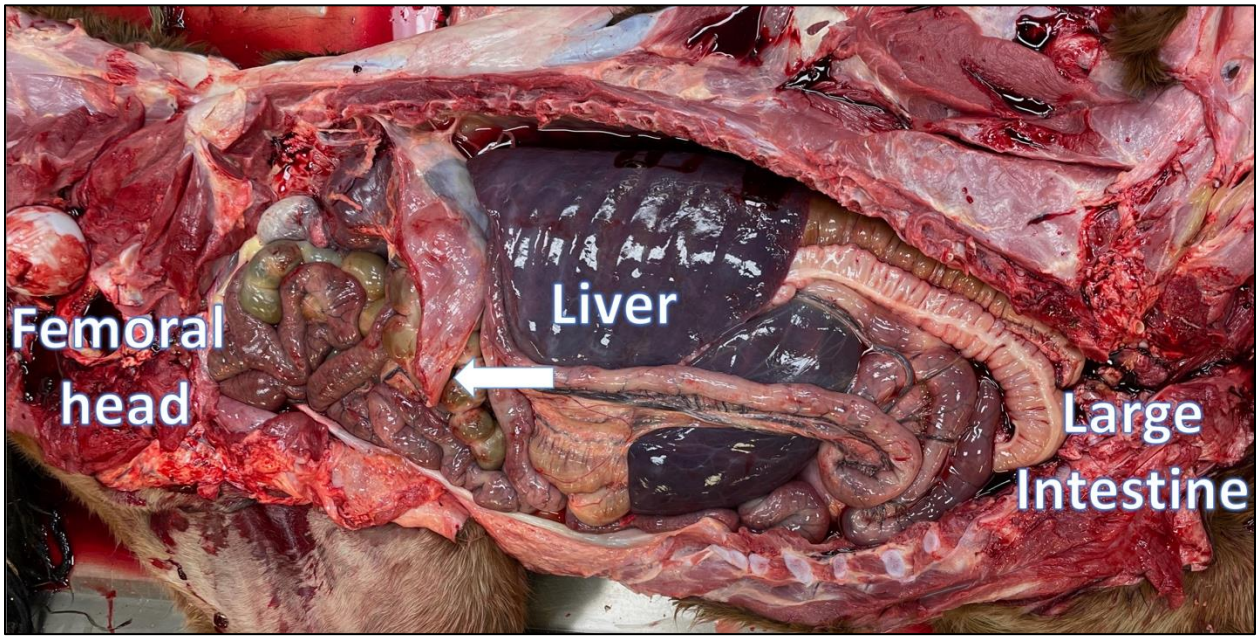

Figure 1. Diaphragmatic defect with abdominal organ herniation in a neonatal foal**.** Edge of diaphragmatic defect (arrow). Lungs and heart are not visible.

Congenital hypothyroidism and dysmaturity syndrome in a Dutch warmblood foal

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AHL Newsletter 2022;26(3):24.

A Dutch warmblood foal was born weak and could not stand. On physical examination, there was marked mandibular prognathism and incomplete ossification of the cuboidal and tarsal bones. The foal was euthanized for suspected congenital hypothyroidism and dysmaturity syndrome.

The mandibular prognathism was notable on postmortem examination with marked protrusion of the mandible, approximately 2.0 cm relative to the maxilla. The lobes of the thyroid gland were also notably enlarged, measuring approximately 6.0 cm x 4.0 cm x 3.0 cm bilaterally. The tarsal and cuboidal bones appeared largely composed of cartilage grossly. Otherwise, no significant gross findings were observed.

On histology, the lobes of the thyroid gland were expanded by hyperplastic follicular epithelial cells forming variably sized follicular structures (**Fig. 1A**), and the bones of the carpus were composed predominantly (>60% of newly formed endochondral trabecular bone) of hyaline cartilage (**Figs. 1B, 1C**). These findings are consistent with hyperplastic goiter (congenital hypothyroidism) and delayed endochondral ossification.

Prolonged gestation, dystocia and retained placenta have been associated with hypothyroidism in affected neonates. Foals are born weak, unable to stand, and often die within days of birth. Grossly in these cases, the thyroid gland is of normal size or mildly enlarged. Thyroid hyperplasia and musculoskeletal deformity (or congenital hypothyroidism and dysmaturity syndrome - CHD) is a recognized syndrome of neonatal foals in North America and Europe. The reported deformities include flexural deformities of the limbs, mandibular prognathism, muscular weakness and delayed endochondral ossification, three of which features were observed in this case.

In animals generally, congenital hypothyroidism is usually a result of inadequate maternal thyroid hormone crossing the placental barrier during in utero development. As a result, the fetal pituitary gland secretes TSH, causing hyperplasia of the thyroid tissue, commonly called goiter. While adult horses are capable of autoregulating thyroid function in the face of abnormal iodine levels, the fetal thyroid gland lacks this ability. Thyroid function will be permanently altered if abnormal iodine intake is present, resulting in congenital hypothyroidism. In the case of CHD, the exact cause is not determined, although dietary causes, toxins and infectious agents have all been suggested. Risk factors for CHD in pregnant mares include ingestion of immature cereal crops, ingesting forages containing nitrites, lack of supplementary minerals (including diets low in iodine), travel during the last 2 trimesters of gestation, and grazing of irrigated pastures.

Prognosis for foals with CHD is generally poor, with euthanasia often elected within several days of birth. The actual underlying cause remains undetected in most cases; therefore, prevention by identifying and resolving specific problems, and provision of supportive care are the only management strategies available at this time. *AHL*

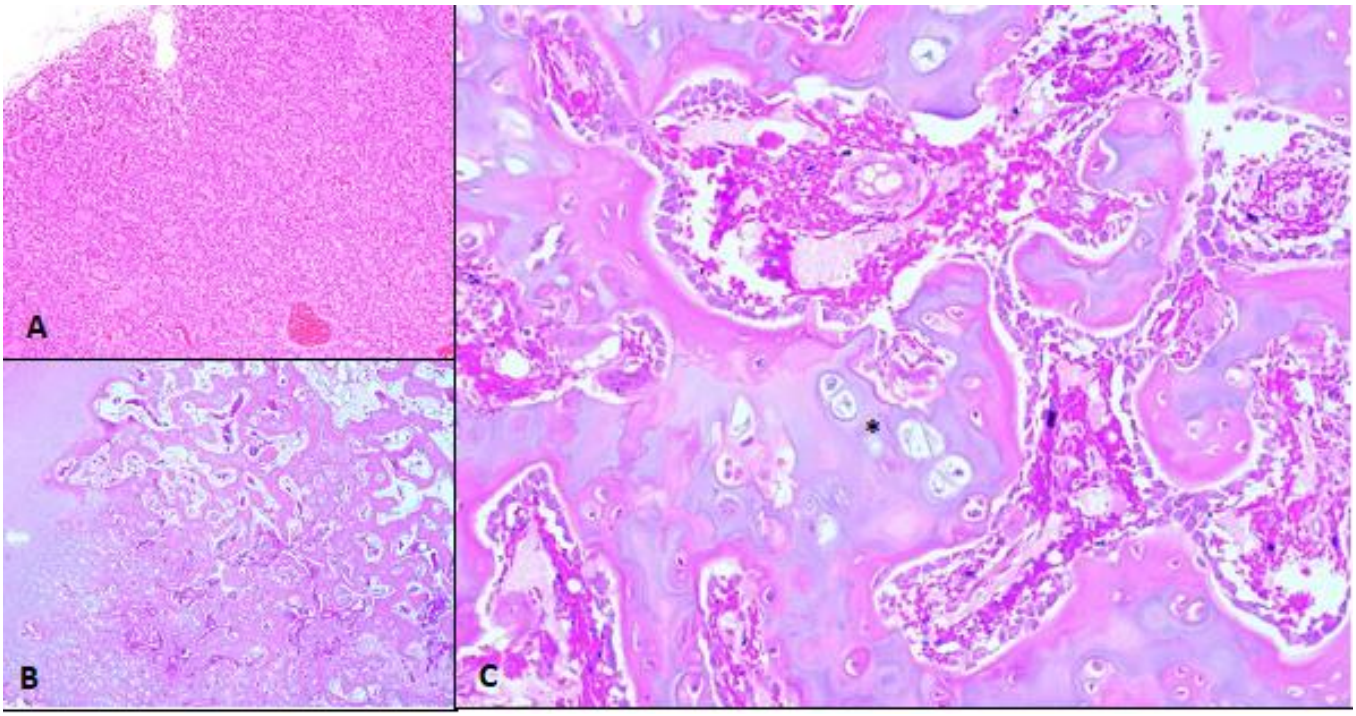

Figure 1. Bone and thyroid gland in a Dutch warmblood foal. **A.** Hyperplastic follicular epithelium in the thyroid gland. **B, C.** Cuboidal bone histology (B - 4x, C - 20x) with incompletely ossified endochondral bone composed of a high proportion of hyaline cartilage (*). H&E stain.

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Salmonella abortion in a dog

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AHL Newsletter 2022;26(3):26.

A three-year-old golden retriever dog was presented to a veterinarian approximately 2 weeks prior to the expected due date, following the onset of labour and subsequent stillbirth of the entire litter. The first stillborn puppy and accompanying placenta were then submitted to the AHL for postmortem examination. Gross examination identified pale mucous membranes, pulmonary atelectasis, and scattered discrete subcapsular foci of congestion in kidneys of the fetus. The placental surface was coated by green-brown-black slightly mucoid exudate, and slight thickening of chorioallantois was appreciable. Microscopically, the placenta contained neutrophilic inflammation accompanied by necrosis, accumulation of fibrin, thrombi, mineralization, and trophoblasts containing intracytoplasmic rod-shaped bacteria (**Fig. 1**). In the fetus, microscopic examination revealed subtle neutrophilic pneumonia and aspiration of amniotic fluid, along with focal renal congestion and extramedullary hematopoiesis in liver. With compatible placental lesions and isolation of high levels of *Salmonella* spp. from the placenta, premature stillbirth was attributed to *Salmonella* infection. Serotyping identified *Salmonella enterica* serovar München. PCR testing of pooled fetal tissues did not detect canid herpesvirus-1 or canine adenovirus-2.

In animals, *Salmonella enterica* serovars can cause clinical disease or may be present and shed from the gastrointestinal tract of asymptomatic carriers. Potential sources of infection include consumption of raw contaminated meat or environmental exposure. In this case, the exact source of *Salmonella* infection was uncertain, as there was no known exposure to raw food or livestock. However, the owner did report that the dog had been found consuming a wild bird a few weeks prior. Thus, it is possible that infection was acquired through exposure to a wild bird harbouring *Salmonella*, with localization of bacteria to the placenta resulting in placentitis and subsequent abortion. Acute clinical salmonellosis typically presents with diarrhea, which can progress to septicemia in some cases, but clinical signs were not reported prior to abortion in this case. *AHL*

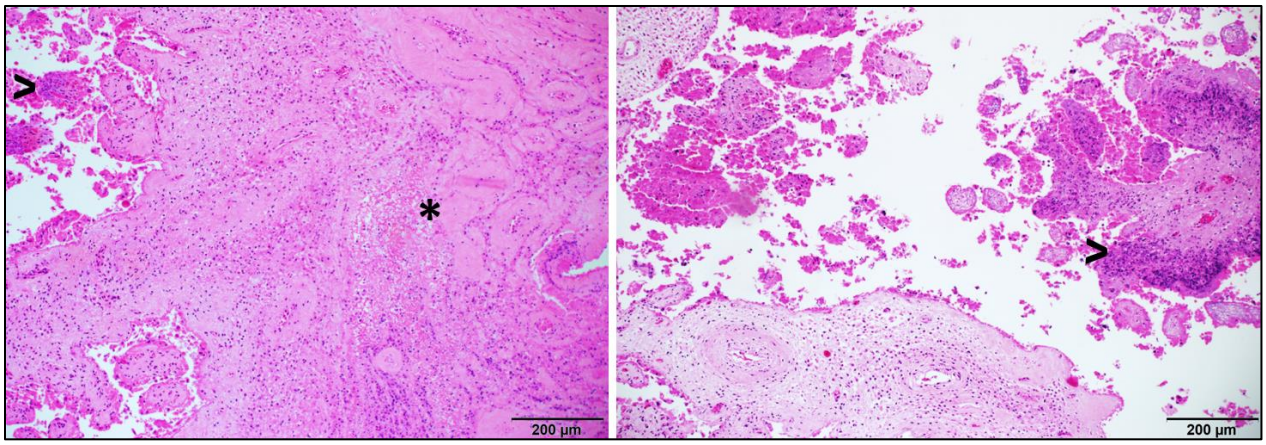

Figure 1. Microscopic section of placenta (10x) containing neutrophilic inflammation accompanied by necrosis (>) and accumulation of fibrin (*****). H&E stain.

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Feline post-anesthetic death: A complication of a rare disease

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AHL Newsletter 2022;26(3):27.

A young male Russian blue cat was presented to the AHL for postmortem following an unexpected death shortly after recovery from neuter surgery. The cat had a long-term history of abnormal gait. Prior to surgery, blood work revealed elevations in AST and ALT. Gross postmortem findings included a severely thickened diaphragm (**Fig. 1A**), and hypertrophy of the muscles of the dorsal neck and shoulders **(Fig. 1B)**. Microscopic review of skeletal muscle tissue from several locations showed myocyte degeneration, necrosis and regeneration with marked variation of myofiber diameter, and extensive endomysial and perimysial fibrosis **(Fig. 2B)**. In addition, some hind limb muscle bundles had massive, widespread myocyte necrosis characterized by sarcoplasmic eosinophilia, fragmentation, vacuolation, and nuclear pyknosis **(Fig. 2A)**. Based on gross and histological findings, a diagnosis of feline muscular dystrophy (also called feline hypertrophic muscular dystrophy) was made.

Feline muscular dystrophy is a very rare condition and there are very few published reports. Depending on the genetic variation, this congenital condition often affects young male cats when X linked. These cats often have a history of limb stiffness or gait abnormalities, and chronic elevations of ALT and AST due to leakage from damaged muscles. Anaesthetic and post-anaesthetic death is well documented with this condition. Dystrophin-deficient cats can develop a "malignant hyperthermia-like syndrome" associated with restraint, stress, or general anesthesia. The massive necrosis seen microscopically in the hind limb skeletal muscles in this case may be representative of this phenomenon.

Confirmation of the type of muscular dystrophy (dystrophin deficient vs. α-dystroglycan deficiency vs. laminin deficiency) requires immunohistochemical/immunofluorescent staining for these skeletal muscle proteins, which was not pursued in this case. *AHL*

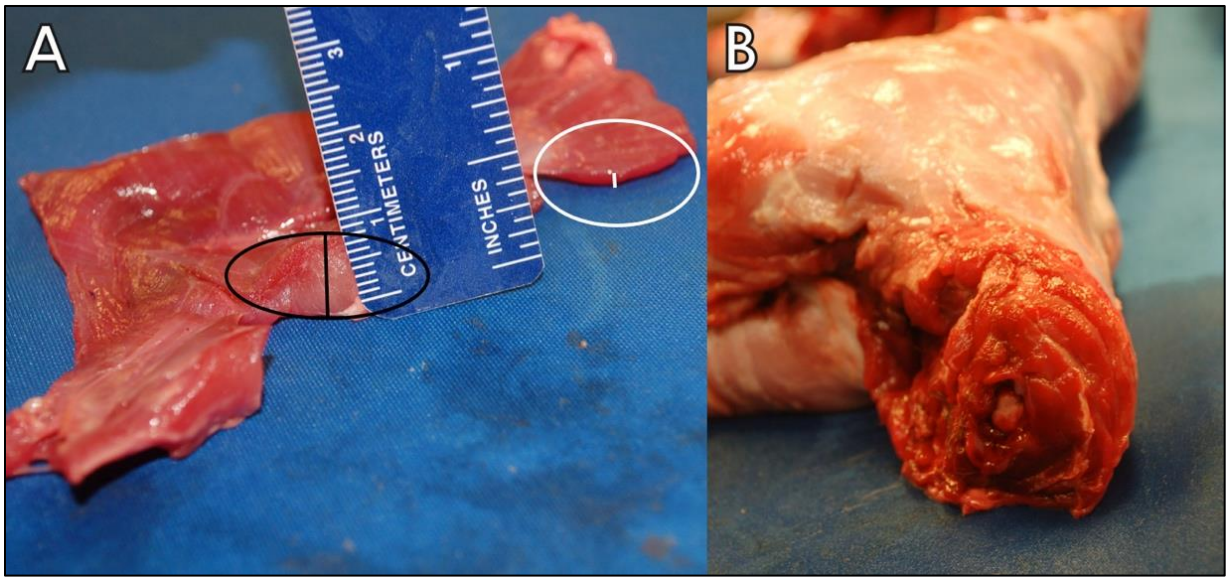

Figure 1. Feline muscular dystrophy. **A.** Severe multifocal thickening of the diaphragm (black line and oval); normal diaphragm thickness (white line and oval). **B**. Hypertrophy of the muscles over the dorsal neck.

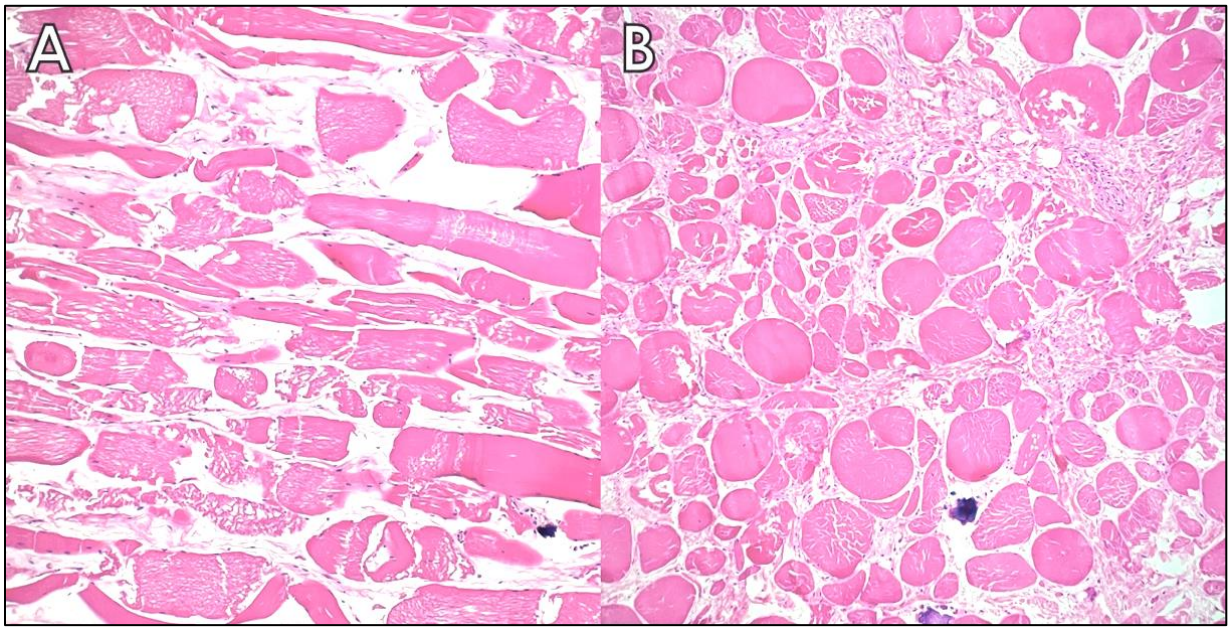

Figure 2. Feline muscular dystrophy skeletal muscle histologic lesions. **A.** Massive hind limb myocyte necrosis characterized by sarcoplasmic vacuolation, fragmentation, hypereosinophilia and nuclear pyknosis. H&E stain. **B.** Marked variation of myofiber diameter with alternating atrophied and hypertrophied fibers, regional endomysial and perimysial fibrosis, and foci of mineralization. H&E stain.

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Osteomyelitis due to *Candida albicans* infection in a dog with previously diagnosed immune-mediated thrombocytopenia

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AHL Newsletter 2022;26(3):29.

A 1.5-year-old, male castrated mixed breed dog with a three month history of immune-mediated thrombocytopenia being treated with combination immunosuppressive therapy was presented with recent onset of lameness and pyrexia which did not respond to antibiotic therapy. Radiographs revealed evidence of aggressive, proliferative bone lesions involving the left scapula, distal humeri, and the right tibia. Direct smears of material aspirated from the humeri and synovial fluid from the tibia were received for cytological evaluation.

Samples from the tibia and right humerus revealed a predominant population of inflammatory cells against a hemodiluted slide background. A large number of neutrophils, moderate number of macrophages and low number of small lymphocytes were also identified. Numerous round to oval yeast that were 5-10 μm in diameter and exhibited a thin clear capsule and granular internal details were scattered amongst this inflammatory cell population, and were also rarely identified within macrophages. Rare narrow-based buds and short hyphae or pseudohyphae were also identified.

Culture of the synovial fluid resulted in a pure growth of *Candida albicans*, as identified by Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) and confirmed by internal transcribed spacer (ITS) sequencing. ITS is a fragment of highly conserved DNA that is used for molecular identification of yeast and fungi.

Candida albicans is a dimorphic fungus that normally resides in the nasopharynx, gastrointestinal tract, and external genitalia of many species, and is opportunistic in causing disease. Intact skin, mucosal barriers and resident microflora are important in preventing *Candida* infections. Thus, anything that disrupts these defenses may facilitate entry of organism into the body. Once in the body, cell mediated immunity is an important determinant of additional spread of infection. Mannan, a *Candida* cell wall glycoprotein, has immunosuppressive properties that facilitate persistent intracellular infection.

Prolonged immunosuppression, cytotoxic chemotherapy resulting in neutropenia, diabetes mellitus, long-term glucocorticoid therapy, and prolonged antibiotic therapy have resulted in an increased incidence of disseminated candidiasis in people. Vertebral infection with *Candida albicans* has been previously reported in a dog, likely secondary to a penetrating wound at that site. Similar to this patient, a favourable response to antifungal therapy was achieved. *AHL*

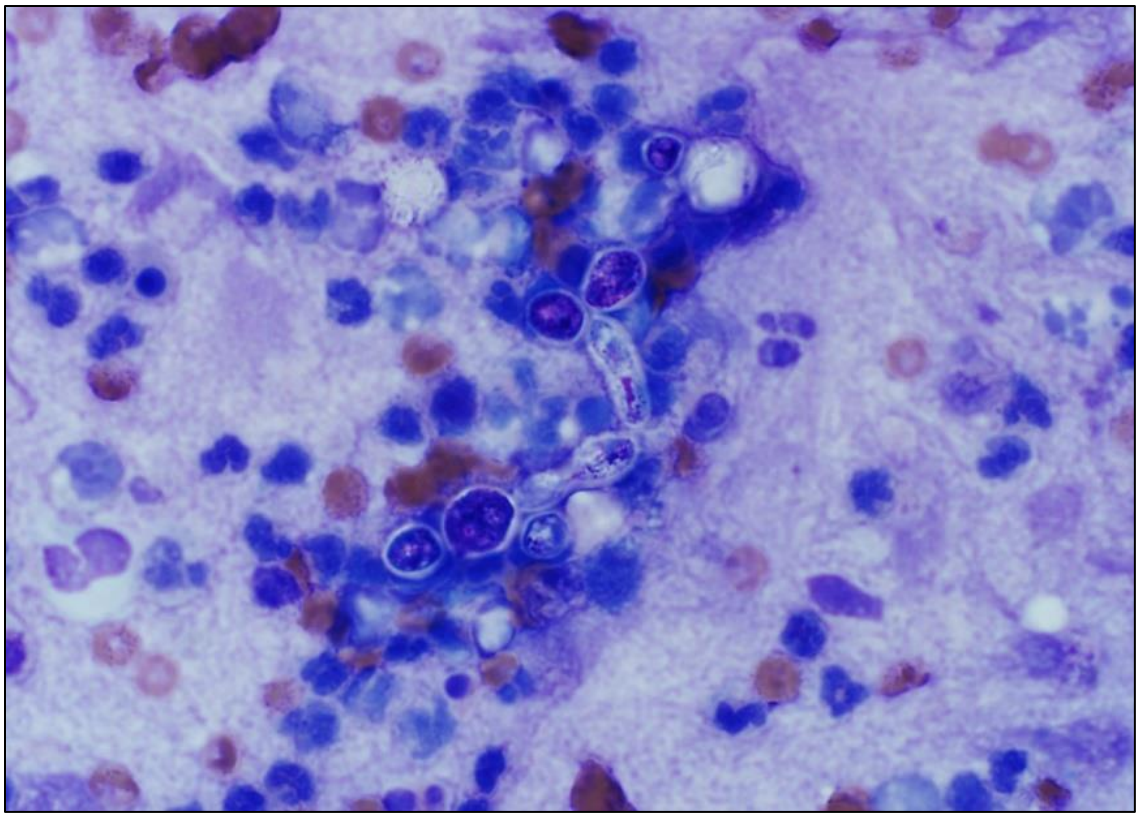

Figure 1. Synovial fluid with yeast and pseudohyphae against an inflammatory cell background. Wright's stain.

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