Laboratory Services Division
Animal Health Laboratory

# AHL Newsletter

**AHL Newsletter, Volume 26, Number 2** **June 2022**

***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.*  *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*

Unfortunately, the prediction of an outbreak of H5N1 highly-pathogenic avian influenza materialized in late March, and the infection has been diagnosed in 20 commercial flocks and 6 small flocks to date, in addition to innumerable wild birds. AHL developed an AI Response Team to manage daily submissions from CFIA staff as they work to identify positive flocks and confirm negative flocks for movement permits. During the first few weeks of the outbreak, the AI Response Team met daily to identify bottlenecks in providing rapid turnaround of test results, and to troubleshoot issues involved in enhanced laboratory biosecurity. Fortunately - due primarily to our dedicated, hard-working staff - AHL has managed to support AI outbreak testing in Ontario successfully, while maintaining business continuity for all of our other, non-avian clients. Cases seem to be slowing down, and it is our hope that the outbreak will resolve soon as the spring migration ends. For a comprehensive overview of wild bird migration and the spread of avian influenza, please check out the article written by Dr. Martin and Dr. Parmley.

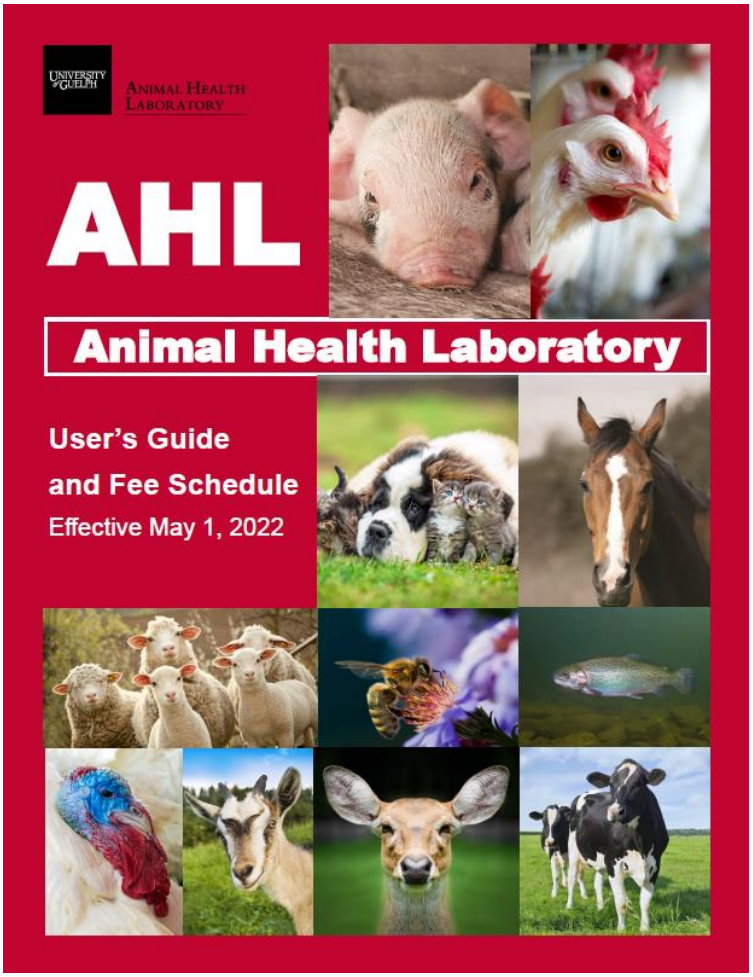
This issue of the AHL newsletter also contains the third section of our antibiotic sensitivity LabNote 64 that describes how to select an appropriate class of antimicrobial drugs for therapeutic purposes. In particular, a list of the drugs on the antimicrobial sensitivity panel that predict susceptibility to other antimicrobial agents is explained by Dr. Slavić. Our updated User's Guide and Fee Schedule was released on May 1 and provides a wealth of detailed information on laboratory tests provided by AHL. While a list of 16 "new" tests is detailed with the ad (next page), many of these are revised tests employing duplex and triplex PCRs that logically group agents and reduce test fees. Always a good thing!

We wish you all good health and a spectacular summer. Please take the time you need to relax and refresh with your families, friends and colleagues. Maybe we'll see each other at a conference or meeting soon.

*Maria Spinato, Director*

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

# AHL User's Guide and Fee Schedule - May 1, 2022




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 2021**:

Bovine adenovirus sequencing
Bovine viral diarrhea virus/Bovine adenovirus/Bovine coronavirus – triplex PCR
Equine adult diarrhea PCR panel
Equine encephalitis virus (EEEV)/West Nile virus (WNV) – duplex PCR
Equine foal diarrhea PCR panel
Fish infectious pancreatic necrosis virus (IPNV) - PCR
*Glaesserella parasuis* - PCR
Hemorrhagic enteritis virus, turkey - ELISA
Infectious bronchitis virus/Infectious laryngotracheitis virus – duplex PCR
Infectious bursal disease virus/Chicken anemia virus – duplex PCR
Influenza A, H1N1/H3N2 typing - PCR
*Lactococcus garvieae* - PCR
Malignant catarrhal fever (OvHV-2) - PCR
Porcine circovirus 1,2,3 – triplex PCR
Porcine circovirus type 3 - sequencing
Serpentovirus (reptile nidovirus) - PCR

# Comprehensive bovine respiratory disease panel - Update

*Jim Fairles*

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AHL Newsletter 2022;26(2):4.

**AHL has adjusted this panel to conform to new test combinations available at AHL**. Many practitioners have asked for complete panels as an aid in diagnostic testing. AHL continues to develop and combine tests to provide more comprehensive diagnostic planning. The comprehensive bovine diagnostic panel has been developed for this reason, and combines several existing tests and test combinations.

The panel **now** includes:

1. Bovine viral diarrhea virus/Bovine adenovirus/Bovine coronavirus – triplex PCR (BVDV and adenovirus typing are available on request.)
2. Bovine respiratory virus PCR panel - BoHV-1 (IBR), BPIV-3, BRSV
3. *Mycoplasma bovis* PCR
4. Bacterial culture, food/fiber producing animals (other than swine).

Samples required:
A) For postmortem samples – **lung** is the required sample
B) For the live animal, there are four choices:

1. Nasal swab (BacT swab for culture, Virus Transport Media (VTM) swab or dry swab for PCR)
2. **Deep pharyngeal swab (normally guarded uterine swab – for bacteriology into gel media; for PCR into VTM or dry swab)**
3. Bronchoalveolar lavage (BAL)
4. Transtracheal wash (TTW)

Note: separate samples are needed for both bacteriology (gel media) and PCR (VTM or dry swab). The swabs cannot be transferred into alternate media upon arrival at the lab.

A reference article (1) outlines a comparison of sample types. There is good agreement when samples types are compared to TTW except for bovine coronavirus (BCV) and BRSV. **Deep pharyngeal swab appears to be the best non-invasive method for sampling**. It is also important that a representative

number of affected animals are sampled to provide a good cross-section of the problem. The actual number needed is always centered around a representative sample and cost of testing.

**This panel is intended for individual animal testing only. If pooled PCR testing is desired for the virus testing (up to 5 samples), each test must be ordered individually. Pooled testing is not available for bacterial culture, as these samples are subject to contamination and pooling will amplify this problem.**

**Reference**

1. Doyle D, et al. Agreement among 4 sampling methods to identify respiratory pathogens in dairy calves with acute bovine respiratory disease. J Vet Intern Med 2017;31(3):954–959.

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## Check out the other AHL comprehensive test panels:

**Bovine neonatal enteric panel bentpnl**

- includes Bovine coronavirus (BCoV) - PCR, Rotavirus ruminant Group A and B - PCR (rocopcr), Sucrose wet mount (sucwt), Bacterial culture, food/fiber producing animals (other than swine) (cultf, bsetup).
- Feces. Collect and split into 3 leakproof sterile containers. Refrigerate immediately.

**Equine adult diarrhea PCR panel adpcrp**

- For adult horses greater than one year of age.
- Includes testing for *Salmonella* spp., *C. perfringens* enterotoxin, *C. difficile* toxin B, Equine coronavirus, *Neorickettsia risticii* and *N. findlayensis*.
- 15 g feces. Collect and split into 3 leakproof sterile containers. Refrigerate immediately.

**Equine foal diarrhea PCR panel fdpcrp**

- For horses less than 1 year of age.
- Includes testing for *Salmonella* spp., *C. perfringens* enterotoxin, *C. perfringens* beta-2, *C. perfringens* netF, *C. difficile* toxin B, *Lawsonia intracellularis*, *Rhodococcus equi*, Equine coronavirus, Equine rotavirus, *Neorickettsia risticii*, and *N.findlayensis*.
- 15 g feces. Collect and split into 3 leakproof sterile containers. Refrigerate immediately.

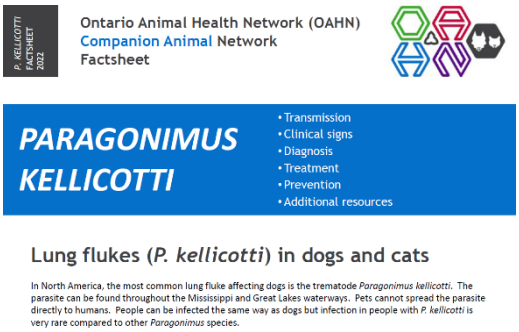
We are also glad to discuss any testing questions and concerns. Please feel free to reach out! ahlinfo@uoguelph.ca or jfairles@uoguelph.ca; 519-824-4120 extension 54530.

# OAHN Update – June 2022

*Mike Deane, Tanya Rossi*

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

This spring, the Ontario Animal Health Network has focused on spreading educational tools, news, and resources about Avian Influenza in Ontario, and working on our upcoming Varroa Monitoring Campaign (with OMAFRA, U of G, and the OBA). In addition, we have finished a research project, created some resources, and held our quarterly species-specific meetings. The resultant veterinary and owner/producer reports 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**

- Ontario Aquaculture Water Quality reports
- Infosheet: *Paragonimus kellicotti* for veterinarians
- Highly Pathogenic Avian Influenza Resources 2022
- Updated Anti-Parasitics Tables for Veterinarians and Owners

**Projects**

- OAHN Companion Animal Research Project: Prevalence of selected pathogens in dogs recently imported from Asia.
- Refining the standard bioassay method for detecting resistance in populations of Varroa mites by acute toxicity

**New Reports**

- Surveillance: Q4 2021 Animal Health Laboratory Data
- Report a Bovine Disease to OAHN
- Salmonella Dublin Update
- Case Report: Salmonella Dublin in a Lactating Dairy Herd
- Practitioner Inquiry: Is leptospirosis making a comeback?

- Outbreak of Highly Pathogenic Avian Influenza (HPAI) in Ontario: H5N1
- Poultry Veterinary Survey Highlights – Q1 2022

- Disease Discussion
- Laboratory Diagnostic Reports
- Slaughter Statistics
- CanSpotASF Surveillance Update
- OVC Research Update

- Fecal Water Syndrome – what's new?
- eNAD / EDM
- OAHN Q4 2021 survey: Key results
- Network member reports
- Laboratory data – Highlights for Q4 2021

- OAHN survey: GI disease, heartworm
- Imported Asian dog study summary
- Rabies: 2nd imported rabid dog
- Lyme disease risk area map 2022
- OAHN info sheets, antiparasitics

# Antimicrobial susceptibility testing: Part 3 – Selection of antimicrobials

*Đurđa Slavić*

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AHL Newsletter 2022;26(2):8.

When performing **a**ntimicrobial **s**usceptibility **t**esting (AST), all diagnostic labs in North America are expected to follow the standards and guidelines established by Clinical Laboratory Standard Institute (CLSI). The goal of the institute is to promote accurate and reproducible AST results, as well as appropriate reporting and interpretation by standardizing all aspects of AST. A subcommittee on veterinary antimicrobial susceptibility testing (VAST) establishes guidelines for veterinary laboratories that include: testing methods, bacterial density, media and drug types, the drug dilutions, incubation times, QC requirements, and - most importantly - interpretative criteria. Previously, AHL published information about antimicrobial susceptibility test methods and selection of bacterial organisms for testing. In this third article of this series, selection of antimicrobials is discussed. The full document is available on AHL website as LabNote 64: https://www.uoguelph.ca/ahl/ahl-labnote-64-antimicrobial-susceptibility-testing-ast

Selection of antimicrobials:

1. Ideally, only one representative of the drug category which has similar efficacy as the other drugs in the same group needs to be tested. Table 1 indicates which drugs are typically tested on behalf of the entire group, and which drugs have to be tested individually.
2. Only non-proprietary or generic drug names are used.
3. Preferably, drugs should be approved for use in specific animal species and for specific disease processes.

**Table 1.** Drugs on AST panel that predict susceptibility to other antimicrobial agents

| ***Drugs*** | ***Predicts susceptibility to*** |
|---|---|
| *Ampicillin* | *Amoxicillin*<br>*Amoxicillin-clavulanate and amoxicillin-sulbactam (only if ampicillin is S*)* |
| *Cephalexin* | *1st generation cephalosporins* |
| *Cephalothin* | *1st generation cephalosporins* |
| *Clindamycin* | *Lincomycin* |
| *Gentamicin* | *All aminoglycosides, except streptomycin* |
| *Tetracycline* | *Doxycycline, minocycline, and oxytetracycline (only if* |

| | |
|---|---|
| | *tetracycline is S*)* |
| *Trimethoprim-sulfamethoxazole* | *Trimethoprim-sulfadiazine* |
| *Sulfisoxazole* | *Sulfonamides* |
| *Erythromycin* | *Azithromycin and clarithromycin (only if erythromycin is S*)* |

*Reprinted with permission from Clinical and Laboratory Standards Institute from: CLSI. Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings. 1st ed. CLSI report VET09. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.*

**S=susceptible. If results for these drugs are R (resistant), then susceptibility to the other specified drugs cannot be predicted and they have to be tested individually*. *AHL*

# *Vascular lesions in a young dairy heifer with BVDV*

*Amanda Mansz, Emily Brouwer*

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AHL Newsletter 2022;26(2):10.

Following the death of an 8-month-old Holstein who had been repeatedly treated for a three-month-long history of pneumonia, a range of formalin-fixed and fresh tissues were submitted to the AHL. Fixed tissues were processed for histopathology and fresh tissues were forwarded for the bovine respiratory viral panel (including PCR for Bovine herpesvirus 1, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus) and Bovine viral diarrhea virus (BVDV) real-time PCR.

Various test methods, including virus neutralization, PCR and immunohistochemistry (IHC), are available for detecting BVDV in both acutely-infected and persistently-infected (PI) animals on an individual or herd level. In this case, the real-time PCR test for BVDV type I was positive (Ct of 28.7; vaccine status unknown) and there was strong positive detection of BVDV antigen by immunohistochemical staining in various tissues including lung, heart, kidney, and liver. Histological lesions of vasculitis with a mild mononuclear cell infiltrate in multiple organs also supported a diagnosis of BVDV infection. A surprising and interesting microscopic feature in this case was the unusual appearance of several arteries within the myocardium (**Fig. 1A**). These arteries had a "renal glomerulus-like" appearance with occlusive vascular wall thickening and hypercellularity. Immunohistochemical staining for viral antigen was also detected within the cells forming these vascular lesions (**Fig. 1B**). PCR results for the bovine respiratory panel viruses were negative.

A single case report has been published describing similar vascular lesions in myocardial and adrenal tissue in a 2-year-old PI steer as a potential result of chronic BVDV infection (1). The paper describes an intraluminal proliferation of partially occlusive "glomerulus-like" spindle cells confirmed to be of endothelial and smooth muscle origin by IHC. Many of these spindle-shaped cells also had positive immunoreactivity for BVDV antigen.

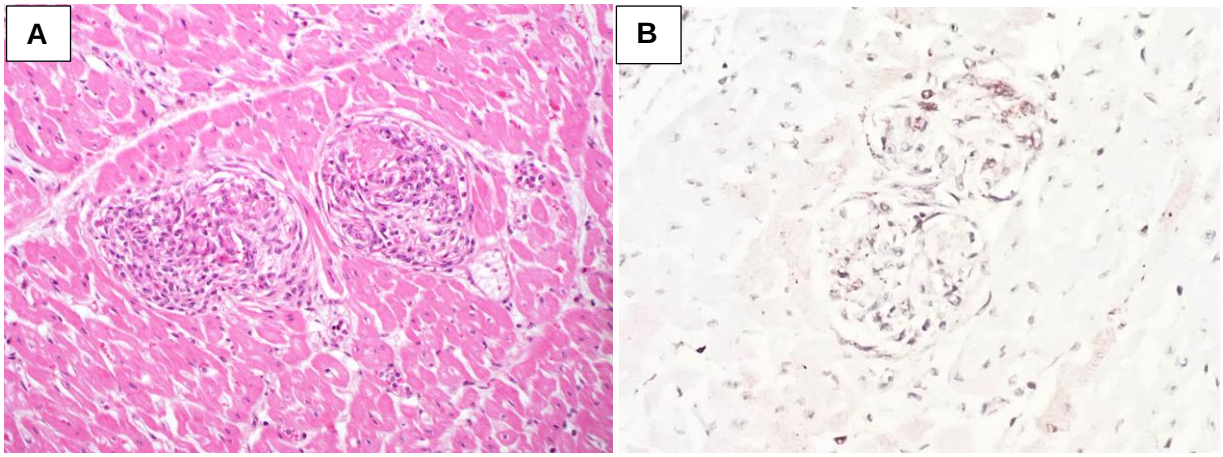


**Figure 1.** Vascular lesions in the myocardium**. A.** Arterial "glomerulus-like" appearance with occlusive vascular wall thickening and hypercellularity. H&E stain. **B.** Positive immunostaining for BVDV. IHC

Although there are no published data definitively confirming BVDV as causation for this type of vasculopathy, the similarities between these two cases of BVDV-infected cattle present an interesting comparison.

For more information, see AHL LabNote 1: Summary of bovine viral diarrhea virus (BVDV) testing at the AHL. *AHL*

**Reference**

1. Breshears MA, et al. Systemic reactive angioendotheliomatosis-like syndrome in a steer presumed to be persistently infected with bovine viral diarrhea virus. Vet Pathol 2008; 45(5):645-649.

# Leptospirosis in an Ontario swine herd

*Margaret Stalker*

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

AHL Newsletter 2022;26(2):12.

Kidneys from several finisher pigs were presented for histologic examination. The kidneys had gross lesions affecting much of the renal parenchyma, varying from coalescing 0.5-1.0 cm pale/white foci to pinpoint raised well-demarcated cortical nodules with a thin red rim, to flat, 1-2 cm geographic red areas extending from the cortex into the medulla (**Fig. 1**).

On histologic examination, all kidneys had varying degrees of chronic and active tubulointerstitial nephritis with interstitial fibrosis, tubular atrophy, lymphoplasmacytic inflammation and formation of discrete interstitial lymphoid nodules (**Fig. 2A**), as well as neutrophilic infiltrates within tubules and spilling into adjacent interstitium. PCR run on renal tissues for *Leptospira* spp. was positive, with cycle thresholds ranging from 25.87 to 33.83. Further genotyping based on single-nucleotide polymorphism (SNP) analysis of the SecY gene was compatible with *Leptospira pomona*. Immunostaining for *Leptospira* spp. was also positive (**Fig. 2B**), highlighting bacteria of typical elongate curving morphology, occasionally with hooked ends, within tubular lumens.

Swine are considered the reservoir / maintenance host for *Leptospira interrogans* serovar *pomona*, in which the organism resides in the proximal convoluted tubules of the kidney. Transmission is direct, through mucosal or skin contact with urine or post-abortion discharges. Only a small proportion of infected animals develop clinical illness, usually only transient mild fever, anorexia and depression during the leptospiremic phase as the bacterium spreads to the liver, kidney, lungs and other organs. Antibody responses clear the infection from most organs, however, the renal tubules are an immunologically privileged site, and establishment of persistent renal infection is the source of ongoing herd maintenance and exposure. The more characteristic signs of *Leptospira* infection within the herd are abortion or premature delivery of pregnant sows, typically litters born 1-3 weeks prematurely with stillborn and weak live piglets.

Since 2008, pathologists at the AHL have identified 9 cases of swine reproductive disease/abortions putatively associated with *Leptospira* infection, so it appears to be uncommon. Prior to introduction of *Leptospira* PCR testing in 2017, this was based solely on interpretation of *Leptospira* serology/MAT titres on sow serum and fetal thoracic fluids. We even more rarely see/recognize the renal lesions in our caseload, although according to the literature, most chronic interstitial nephritis lesions observed in finisher swine are thought to be leptospiral in origin (1). A review of 230 *Leptospira* PCR tests run on porcine submissions since 2017 revealed only a single positive result in placentas from a group of aborted fetuses, prior to the PCR-positive kidney samples tested from this same herd.

Leptospirosis is an important zoonotic illness, and humans (and other incidental hosts) can be infected indirectly by exposure to environments contaminated by the infected urine of carrier animals. Such exposures can be an occupational hazard for both producers and veterinarians. *AHL*

**Reference**

1. Cianciolo R, Mohr FC. Urinary System. In: Jubb, Kennedy & Palmer's Pathology of Domestic Animals, 6th ed. Maxie MG, ed. Elsevier, 2016; vol 2:433-438.

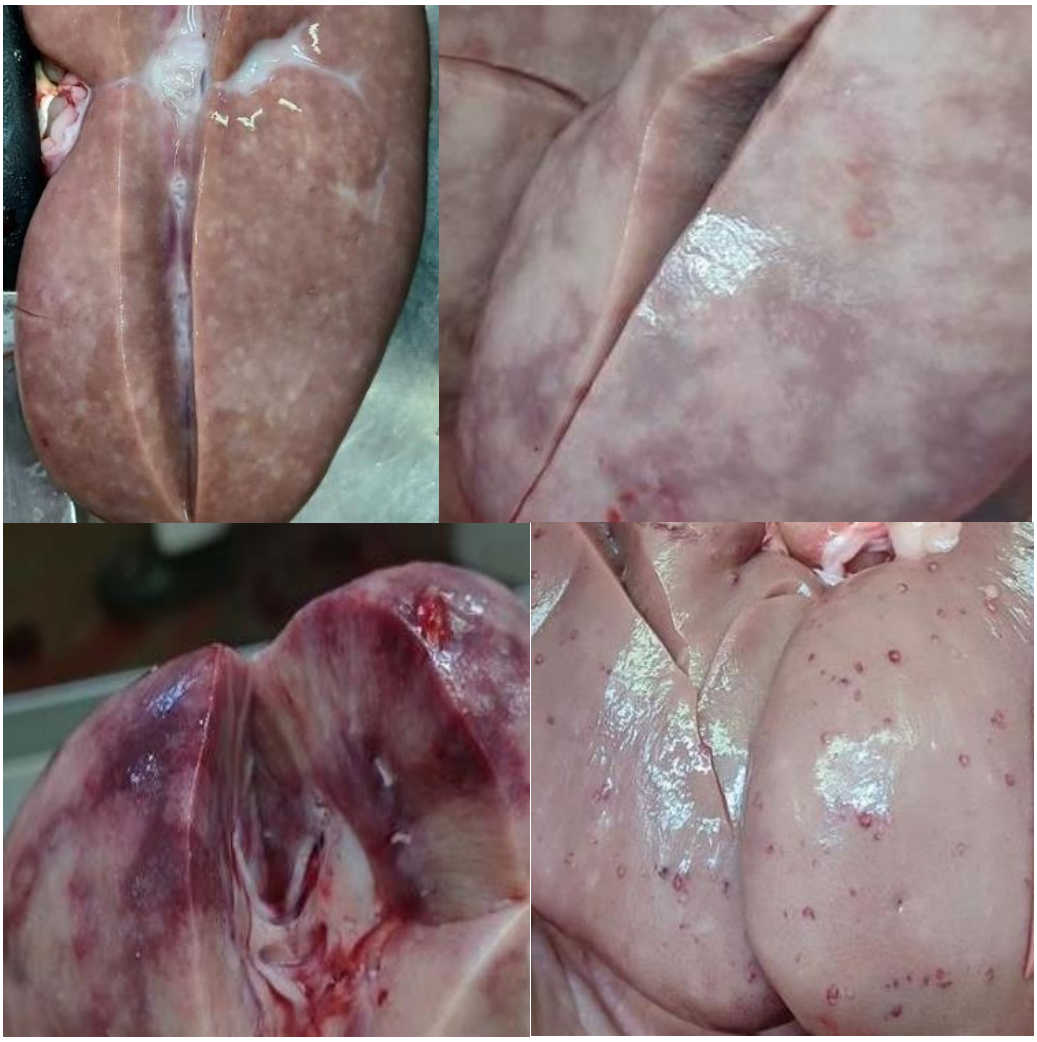

**Figure 1**. Various gross presentations of kidneys from finisher pigs with renal leptospirosis.

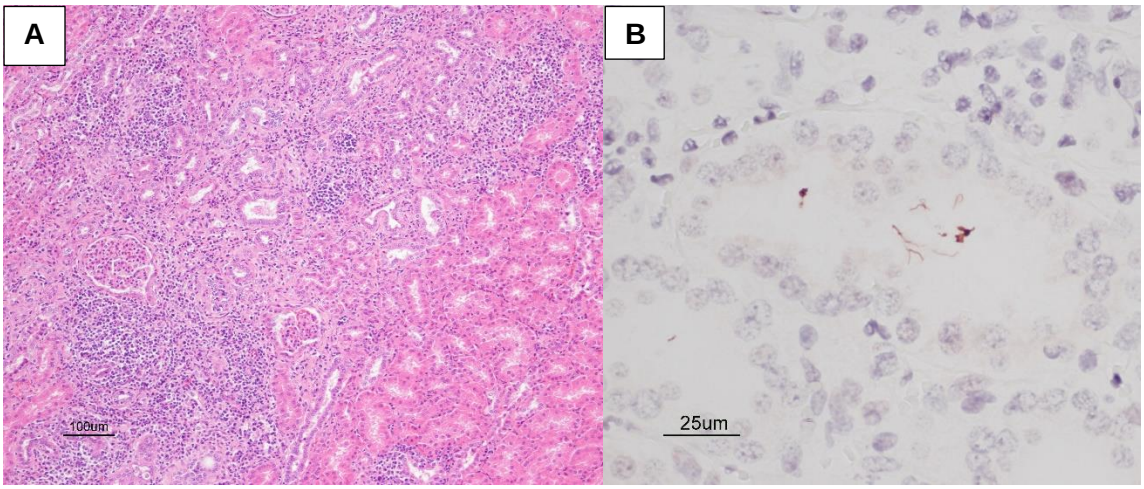


**Figure 2**. Histologic sections of renal leptospirosis. Kidney with tubulointerstitial nephritis (A). H&E stain. Immunostaining highlights filamentous leptospires within a tubular lumen (B). IHC

# Wild bird migration and the movement of Avian Influenza virus

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AHL Newsletter 2022;26(2):14.

Avian Influenza (AI) is caused by type A influenza virus (family *Orthomyxoviridae*, genus *Influenzavirus A*) and is classified into subtypes by 2 surface proteins: hemagglutinin (H1-H16) and neuraminidase (N1-N9). As an RNA virus, errors are made during replication that cause the virus to change and evolve. Sometimes these errors are minor, but other times larger pieces of genetic material can be traded with another AIV virus, increasing the risk of the virus becoming highly pathogenic (causing severe disease in poultry) or able to infect mammals (i.e., swine, humans). Avian Influenza Viruses (AIV) can be grouped as low-pathogenic (LPAIV) or highly-pathogenic (HPAIV, H5 and H7 subtypes). In countries such as Canada where LPAIVs are not endemic and are considered a Foreign Animal Disease (FAD), flocks are destroyed when HPAIV is identified to prevent further spread of the virus. The low pathogenic subtypes of H5 and H7 are handled in the same manner as HPAIV, as these subtypes can transform into HPAI as the disease spreads through the flock(s). In order to manage the risk of poultry flocks, swine or humans contracting this virus, potential risk factors must be considered. One of these risk factors is the wild bird population.

AIV naturally infects wild birds, and most wild bird species usually do not show signs of infection. All known subtypes of AIV have been isolated from wild birds, and LPAIVs are the most common. The virus is carried and replicates in the intestinal and respiratory tracts, spreading via feces, saliva, and nasal secretions. Domestic poultry are highly susceptible to AIVs. If flocks are infected with LPAIV, the birds may have mild signs, but the virus is more likely to transforms into HPAIV. If a flock is infected with HPAIV, the birds tend to have severe clinical signs and high mortality. This has significant impacts including economic loss, international trade sanctions, and potential spread to humans and other livestock.

Since wild birds can carry AIV without clinical signs, they can potentially pass AIV to other wild birds, including during migration. There are 8 migratory pathways worldwide, and many of these overlap (**Fig. 1**). This creates the potential for AIV to emerge and evolve, as well as to move long distances by transmitting the virus to other migratory or non-migratory birds, including poultry. Previous research tracking H5N1 HPAIV between migratory bird flyways identified a correlation between wild bird migration and H5N1 HPAIV infections in people. This suggests that transmission is strongly associated with avian migration. Wild birds are tested annually in Canada for AIVs. Most wild bird samples are collected during the fall and spring migrations, with identification of AIVs being more likely during the fall migration and at higher latitudes. It is thought this pattern of identification occurs due to the larger number of naïve, juvenile birds in the population during the fall migration. As the juveniles fly south, they become infected, recover, and are less likely to be carrying the virus once they reach lower latitudes.

There are certain migratory birds, predominantly ducks, followed by geese and gulls which are mainly responsible for annual movement and evolution of AIV. The virus is stable and can survive in the environment for weeks to months. One study showed that AIV in duck feces remained infective for at

least 30 days at 4 °C and for 7 days at 20 °C. The aquatic environment is also a potential source of infection for other birds or mammals. While research has provided a better understanding of how AIVs move over long distances (i.e., migration pathways), it is still not well understood how AIVs move from wild birds into commercial poultry populations housed in closed barns.

To track the movement of AIVs, the strains are compared by looking at their genetic structure in comparison with other isolates. There is a world influenza database (GISAID – Global Initiative on Sharing Avian Influenza Data) in which the genetic sequences of AIVs can be uploaded and compared to other AIVs. This database can be used to compare AIVs from the same and different geographic areas, to look for mutations, and to determine if there is movement into bird or mammalian populations (i.e., swine or humans). Part of this comparison consists of constructing a phylogenetic (family) tree of the viruses to see how they cluster into groups; viruses that are more closely related will cluster closer together. As more information is gathered, we can have a clearer picture of how AIVs move within and between species of birds and mammals, and how the viruses change over time.

It should be noted that when humans become infected with Influenza A (the same group of viruses as AIV), virus mutations or recombinations can occur, and birds or swine can then be infected with the virus from humans (and vice versa). That is why poultry workers are encouraged to have a 'flu shot' every year to protect themselves and their flocks.

It should also be remembered that AI is not the only FAD that can be transmitted by wild birds. Cormorants play an important role in the maintenance and circulation of Newcastle disease virus (Avian Paramyxovirus-1) in North America, which is also designated a Foreign Animal Disease in Canada. NDV was detected in cormorants in Ontario in 2021.

Please be aware that there is currently a H5N1 HPAI outbreak in North America that has been detected in multiple wild bird species, poultry, and mammals (wild foxes). The strain has been identified as the H5 Gs/GD (Goose Guangdong) Eurasian lineage of HPAI that started circulating in 1996, has genetically evolved over time, and has gained wide geographic dispersion due to asymptomatic infections in migratory aquatic birds. The current strain circulating in North America is causing disease in a wider variety of wild birds, including waterfowl (ducks, geese, swans), shorebirds (gulls), raptors (hawks, owls), and scavengers (turkey vultures). Multiple Canadian provinces have had multiple commercial poultry and small flock poultry identified with H5N1. Current recommendations are to keep domestic poultry, or any captive avian species (i.e., pigeons, pet birds), away from wild birds to prevent transmission of H5N1.

For more information, please refer to the following links:
Canadian Wildlife Health Cooperative (CWHC):
http://www.cwhc-rcsf.ca/avian_influenza.php
CWHC diagnosis of H5N1 in wild foxes:
http://blog.healthywildlife.ca/influenza-a-h5n1-virus-detected-in-wild-foxes-vulpes-vulpes-in-ontario/
Poultry Industry Council (Biosecurity and Disease resource page):
https://www.poultryindustrycouncil.ca/resources/biosecurity-and-disease
Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA):
http://www.omafra.gov.on.ca/english/livestock/vet/facts/avian_influenza.htm
Canadian Food Inspection Agency (CFIA):
https://inspection.canada.ca/animal-health/terrestrial-animals/diseases/reportable/avian-influenza/protect-your-flock/eng/1614352583029/1614352660146?utm_campaign=cfia-acia-avianinfluenza-22-23&utm_medium=sem&utm_source=ggl&utm_content=ad-text-en&utm_term=avian%20influenza&adv=2223-260350&id_campaign=17067478476&id_source=134758936703&id_content=595205628412&gclid=Cj

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*AHL*

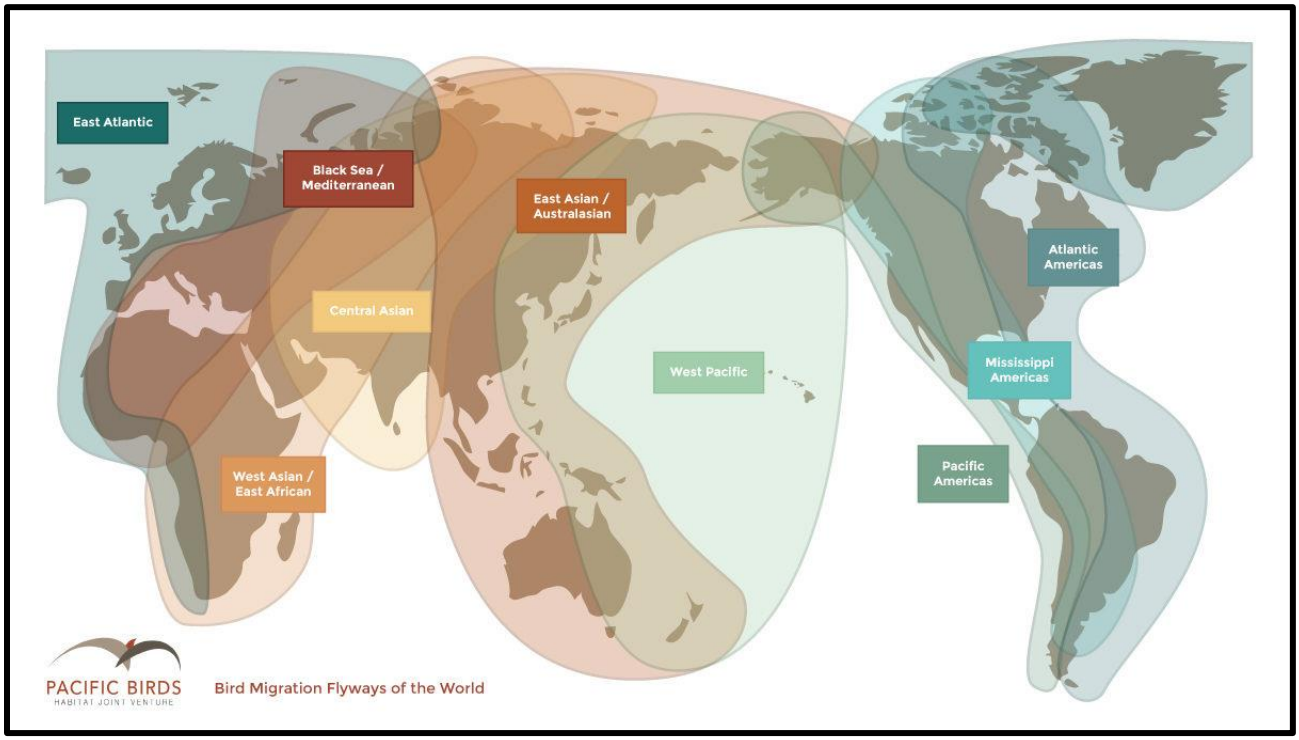


**Figure 1.** The 8 migration pathways. (https://pacificbirds.org/birds-migration/the-flyways/)

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1. Blagodatski A, et al. Avian Influenza in wild birds and poultry: Dissemination pathways, monitoring methods, and virus ecology. Pathogens 2021;10:630.
2. Diskin ER, et al. Subtype diversity of Influenza A virus in North American waterfowl: A multidecade study. J Virol 2020;94: https://doi.org/10.1128/JVI.02022-19
3. Karamendin K, Kydyrmanov A. Cormorants as a potentially important reservoir and carrier of Newcastle disease virus on the Asian continent. Front Vet Sci 2021;8:648091.
4. https://www.ioes.ucla.edu/project/avian-influenza-virus-north-american-migratory-birds/

# Bromethalin exposure in a free-ranging American black bear (*Ursus americanus*)

*Sherri L. Cox, Brian Stevens, Felipe Reggeti*

*National Wildlife Centre and Integrative Biology, University of Guelph (Cox); Canadian Wildlife Health Cooperative (Stevens); Animal Health Laboratory, University of Guelph (Reggeti)*

AHL Newsletter 2022;26(2):17.

A black bear cub (*Ursus americanus*) from Ontario was found minimally responsive and weak. After presentation at a veterinary clinic, blood work results were within reference limits except for a mildly elevated ALT (550 U/L; RI: 11-309 U/L); radiographs were unremarkable. The cub was then transferred to a wildlife rehabilitation center for supportive care, where it developed moderate to severe cerebellar ataxia, inability to control its head movements and self-mutilation of its hind limb. Occasional unilateral epistaxis was reported. Analgesics, anti-inflammatories and antibiotics (for possible *Toxoplasma* infection and the contaminated wound) were prescribed. The ataxia resolved after a week and the bear was doing well; however, unilateral epistaxis worsened and mucous membranes became pale. Blood was collected for follow-up testing but the bear's condition deteriorated and it died soon afterwards. On the antemortem blood sample, coagulation profile was within normal limits, but the CBC showed pancytopenia and mild hypoalbuminemia (23 g/L; RI: 30-51 g/L).

On postmortem examination, the mucous membranes were markedly pale and no abnormalities were noted in the nasal cavity. On histopathology, rare perivascular lymphoplasmacytic cuffs and rare focal gliosis were noted in the cerebral cortex. The bone marrow was hypoplastic (cellularity <10%). Fluorescent antibody testing for rabies virus was negative. Liver samples submitted to the AHL toxicology section tested negative for anticoagulant rodenticides (LC-MS/MS), and no toxins were detected with a comprehensive toxin screen (GC-MS and LC-MS/MS); however, adipose tissue was positive for desmethylbromethalin (LC-MS/MS).

Bromethalin is a potent neurotoxicant with no anticoagulant activity nor known antidote. It is rapidly absorbed in the gastrointestinal tract and widely distributed throughout body due to its high lipophilicity. Clinical signs associated with acute toxicity may occur 4 hours to 7 days after ingestion. Two clinical forms have been described: the initial form consisting of tremors, hyperesthesia, seizures and death; and the paralytic form characterized by depression and disorientation progressing to ataxia and rear limb weakness within 7 days, progressively worsening over 1 to 2 weeks. The mechanism of action consists of uncoupling the oxidative phosphorylation in the mitochondria, resulting in impaired energy metabolism primarily affecting the central nervous system.

The histopathological lesions in this bear were subtle and inconsistent with bromethalin toxicosis (i.e. "spongy degeneration" of the white matter); however, identification of desmethylbromethalin (a highly toxic metabolite of bromethalin) confirmed exposure, absorption and metabolism of the substance. Since the bear recovered from the neurological signs one week after presentation, it was assumed to be exposed to a low (sub-lethal) dose. Considering other significant findings including bone marrow hypoplasia leading to pancytopenia and epistaxis, the cause of death was probably multifactorial.

To reduce the risk of accidental exposure to rodenticides, Canadian regulatory agencies prohibited domestic class products containing second-generation anticoagulants, leading to increased popularity of rodenticides with other active ingredients, such as bromethalin. Since bromethalin-containing rodenticides are easily available (e.g., hardware stores), these products are registered for indoor use only

and must be inaccessible to children, pets, livestock and non-target wildlife, in order to ensure consumers' safety. Unfortunately, accidental exposures do occur. *AHL*

**Reference**

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# Recurring cutaneous melanoma in a South African white lion

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A formalin-fixed excisional skin biopsy from a cutaneous mass obtained from a 7-year-old male South African white lion (*Panthera leo melanochaita*) was received at the Animal Health Laboratory for histopathological evaluation. The mass presented as a 1.0 × 1.0 cm ulcerated region along the dorsal mid-spine. Following surgical excision, a diagnosis of a spindle cell neoplasm with extensive epidermal ulceration was made on histopathology. In addition, a number of unusual perifollicular changes (follicular atrophy and destruction, perifollicular fibrosis, epithelial dysplasia and melanin dispersal) were also noted within the sample, and along with the ulcerated changes, was thought to possibly represent an unusual reaction to excessive self-trauma and habitual licking around the primary tumour site. Although not validated in this species, a number of immunohistochemical (IHC) cell markers (Melan A/PNL2, CD3, CD20, and CD18) were attempted, along with a toluidine blue stain. None of the neoplastic cells and only a few perifollicular mast cells and lymphocytes showed any positive immunoreactivity or staining.

The central mass appeared to be completely excised, however, regrowth was noted at the same site approximately 2 years later. This time, the mass was a darker, black pigmented region with a raised cobblestone surface (**Fig. 1A**) that appeared to be changing more aggressively over the preceding couple of weeks. The mass was again surgically excised and submitted to the AHL for histopathology.
In contrast to the original small, central well-demarcated nodule, multiple occasionally coalescing nodules of short, interlacing streams and bundles of elongate to more plump, almost round spindle-shaped cells were now more extensively distributed throughout the dermis (**Fig. 1B**). Nodules also often crowded around and variably effaced hair bulbs and adnexal structures, and exhibited faint fibrovascular packeting/nesting (**Fig. 1C**). Rare individual deep dermal nodules, as well as some of the smaller and poorly-demarcated peri-follicular aggregates occasionally contained dark brown to black granular pigment (melanin) that obscured the nucleus. Mitotic activity was rare.

Given the rounder and more packeted morphology of tumour cells, as well as the presence of cytoplasmic melanin pigment, a tentative diagnosis of cutaneous melanoma was made. Melan A/PNL2 IHC was performed for further confirmation. Surprisingly, but consistent with that of the initially-excised mass, IHC staining for both markers again proved negative. Distant dermal melanocytes also did not show any immunoreactivity, suggesting that the Melan A/PNL2 antibodies may not cross-react in this specific felid species. As such, another common neural crest origin and melanocyte IHC cell marker, S100, was

performed instead, with the diffuse positive immunoreactivity of the neoplastic cells ultimately supporting a diagnosis of cutaneous melanoma in this lion (**Fig. 1D**).

Although melanoma is a fairly well-known entity in domestic cats, it has only rarely been reported in large non-domestic felids, with only four such reports being present in the primary literature (one Siberian tiger and three African lions). In the African lion cases, one mass was located intra-ocular, another within the haired skin along the mandibular lip, and the third along the pinna. Metastatic disease was reported in two of these cases and as such, could also represent a possible concern for this lion. Interestingly, one of these case reports also involved a white lion. This does raise the question of whether or not leucism (which is due to partial loss of pigmentation rather than complete absence of melanin in the case of albinism) in conjunction with environmental UV light exposure could represent a possible predisposing factor for the development of cutaneous melanoma in this subspecies of lion. Although the sample size of white lion cases here is far too low to make any significant conclusions, similar associations have been recently reported in the paler-coated golden and king colour variant wildebeest (*Connochaetes taurinus*) (n=4).

This lion did very well throughout recovery and the excision site healed uneventfully during the subsequent weeks. To the authors' knowledge, the lion continues to do well, and there have not been any signs of reoccurrence at the excision site, metastasis or additional lesions arising elsewhere. Consistent and diligent monitoring is ongoing to provide the best quality of life possible. *AHL*

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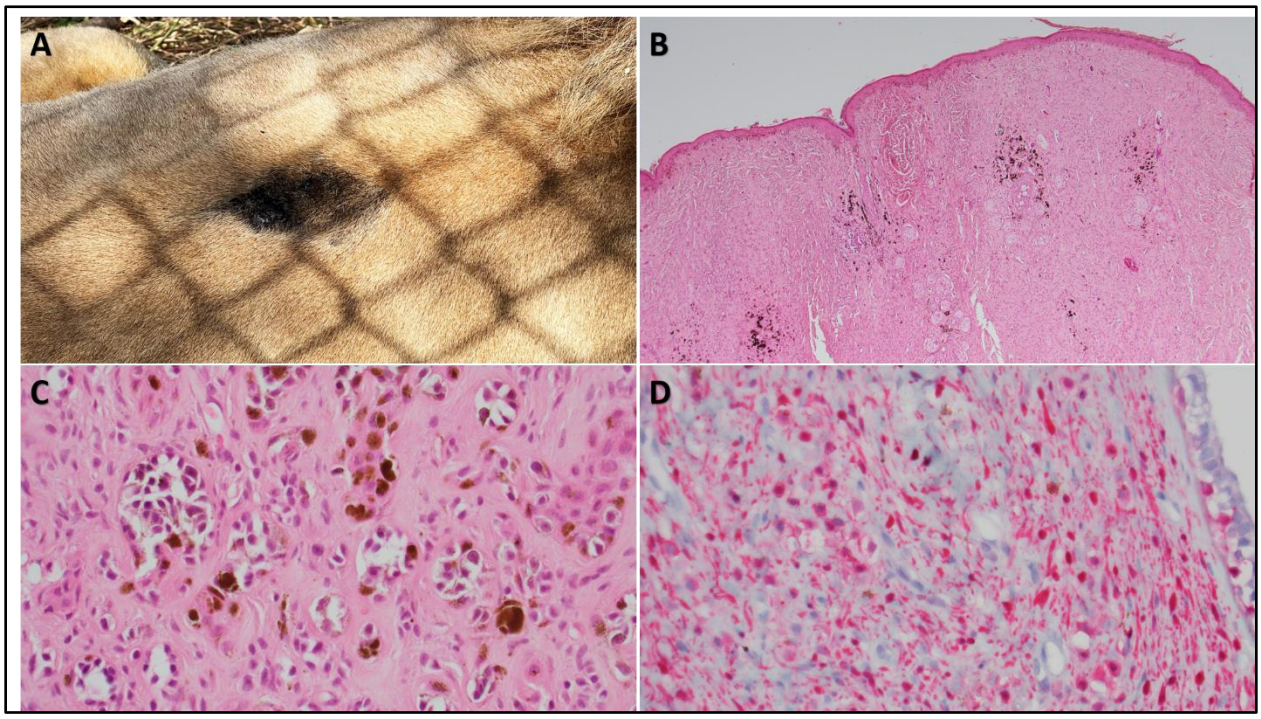


**Figure 1.** Cutaneous melanoma in a male South African white lion. **A.** Regrowth of the mass at the original excision site showing dark black pigmentation and a raised cobblestone surface. **B. Skin, H&E**

**stain 4x.** The dermis contains multiple coalescing nodules that often crowded around and variably effaced hair bulbs and adnexal structures. **C. Skin, H&E stain 40x.** Neoplastic cells are composed of short, interlacing streams and bundles of elongate to more plump, almost round spindle-shaped cells that occasionally exhibited faint fibrovascular packeting/nesting and contain variable amounts of dark black cytoplasmic melanin pigment. **D. Skin, S100 IHC stain 40x.** Neoplastic cells exhibit strong positive nuclear and cytoplasmic immunoreactivity, consistent with a melanocytic origin.

# Extra-osseous osteosarcoma in a dog

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A five-year-old, female spayed Labrador dog was presented for evaluation of a 3 cm firm, subcutaneous mass on the caudal aspect of the left shoulder. The mass was first noted two months previously, and fine needle aspiration at that time provided a diagnosis of soft tissue sarcoma. The mass had undergone recent, rapid growth to its current size, but did not appear to be affixed to deep tissues. The dog was otherwise clinically well.

The mass was re-aspirated and slides submitted for cytological evaluation. Cellular composition varied from slide to slide. Occasional slides contained a predominance of individual round cells consistent with an osteoblast origin; these cells exhibited round to oval, centrally to eccentrically placed nuclei with variable numbers of small nucleoli and moderately abundant and basophilic cytoplasm. Intermixed with this round cell population were occasional large fusiform multinucleated giant cells consistent with an osteoblast origin. The remaining slides contained clusters of pleomorphic fusiform spindle cells embedded in variable amounts of brightly eosinophilic extracellular matrix (interpreted as osteoid), and intermixed with both osteoblasts and osteoclasts **(Fig 1)**. A cytological diagnosis of sarcoma, most consistent with an extra-osseous osteosarcoma (ExOSA), was made. Prior to surgical excision, a CT scan was performed and a subcutaneous location to this mass was confirmed, with no underlying bone involvement identified **(Fig 2)**. Whole-body CT showed no evidence of other lesions.

Histological evaluation of the excised mass and surgical margins revealed a well-demarcated, encapsulated expansile mass in the deep subcutis. The neoplasm was composed of spindle cells arranged in streams, bundles, and whorls on a fibrovascular matrix which occasionally contained embedded foci of eosinophilic extracellular matrix, consistent with osteoid **(Fig 3)**. Occasional multinucleated giant cells consistent with osteoclasts were noted. The inked surgical margins did not contain any neoplastic tissue. A histological diagnosis of extra-osseous osteosarcoma, fibroblastic, productive, was made. ExOSA is a malignant, osteoid-producing stromal neoplasm without primary periosteal or bone involvement. Although less common than its skeletal counterpart, ExOSA are occasionally reported in dogs and cats, with the mammary gland representing the most common location. Various other visceral and soft tissue sites have been reported (e.g., muscle, eye, thyroid gland), with neoplasms also noted secondary to injection site reactions, trauma, granulomas (e.g., *Spriocerca lupi* infection), and foreign bodies. ExOSA are often grouped along with visceral soft tissue and mammary osteosarcomas, both of which tend to have a poor prognosis. Information on the specific prognosis of skin/subcutaneous osteosarcoma in dogs is limited. One relatively large study included 13 dogs with ExOSA at these sites, and reported median survival times from 240 to 486 days. Although the mass described in this report appears to have been completely excised with clean surgical margins, the prognosis for this patient is unknown. *AHL*

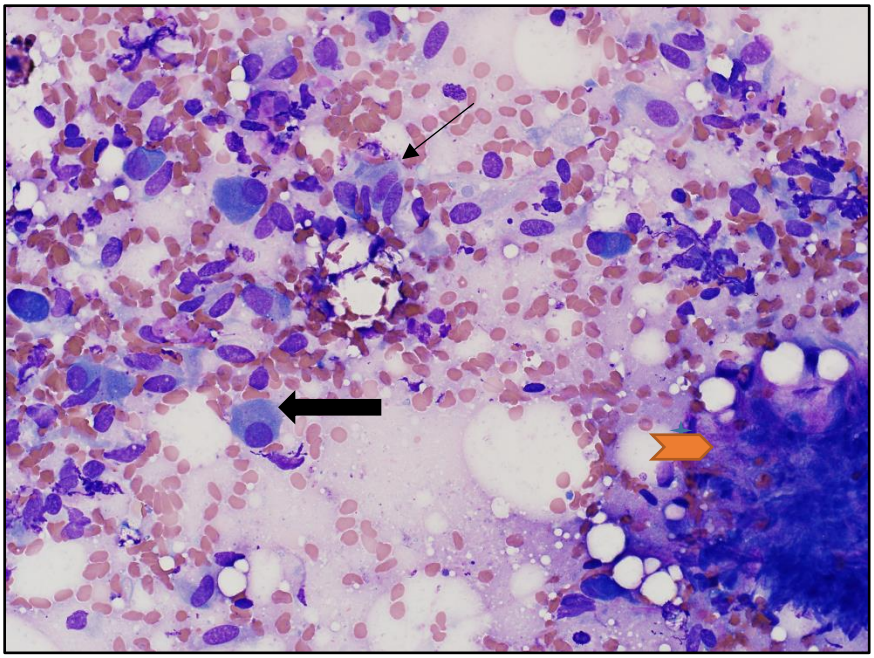

**Figure 1.** Cytological features of the subcutaneous mass including stromal cells (slender arrow), osteoblasts (thick arrow), and stromal cells embedded in osteoid (yellow arrow). Wright's stain.

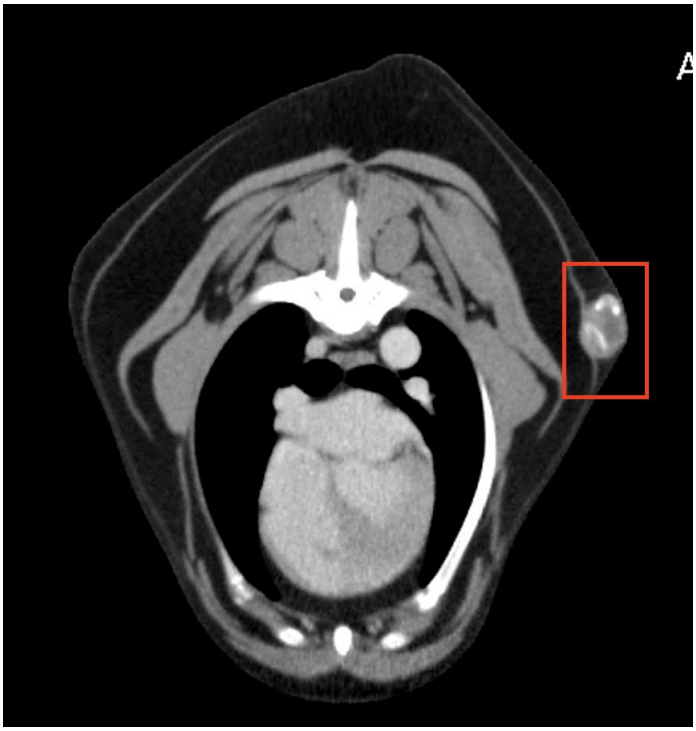

**Figure 2.** CT image showing a well delineated, soft tissue and mineral density mass (red rectangle) that is mildly heterogeneously contrast enhancing on the lateral aspect of the left thorax in the subcutaneous fat, caudal to the left scapula.

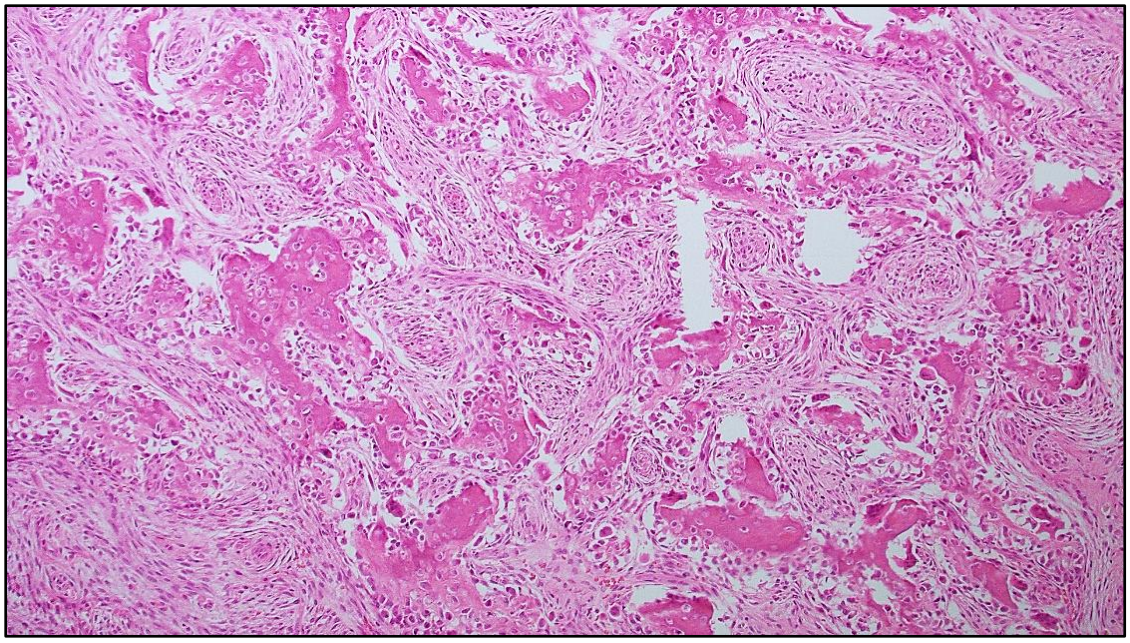

**Figure 3.** Histological features of the excised mass illustrating plump spindle-shaped cells arranged in interlacing streams, bundles and whorls with foci of eosinophilic matrix (osteoid). H&E stain.

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# Fatal heartworm (*Dirofilaria immitis*) infection in a dog with evidence of cerebral nematodiasis

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A two-year-old, German Shorthair Pointer presented with acute onset circling to the left, mild ataxia, salivation, moderate tachypnea and tachycardia. The vaccine history (DHPP and rabies) was up to date. The patient died after failing to respond to a short course of NSAIDs and antibiotics.

On gross postmortem examination, the lungs were mottled red, poorly aerated, and there was a focal depressed area of pleural fibrosis. The liver was enlarged, firm, and mottled red, with fine strands of capsular fibrin (passive congestion). When the heart was dissected, multiple, long thin white worms were present in the pulmonary artery and right ventricle. Moderate numbers of intestinal worms (ascarids) were also identified. The brain was grossly unremarkable.

Histopathology revealed pulmonary edema, hemorrhage and occasional pulmonary thrombosis. In the grossly fibrotic area of the lung, there was localized endarteritis with periarterial granulomatous inflammation compatible with heartworm, although no parasites were captured in section (**Fig 1**). There was acute centrolobular hepatic necrosis in the liver, compatible with acute passive congestion and attributed to pulmonary hypertension/right sided heart failure secondary to infection with heartworm. Neurologic signs were attributed to small multifocal areas of thrombosis and hemorrhage in the cerebral cortex and a focal granuloma in the midbrain, presumed secondary to aberrant visceral larval migrans of nematodes, either heartworms or ascarids (**Fig 2**).

Fewer than a dozen fatal cases of advanced canine heartworm infection have been submitted to the AHL between 2012-2022. All dogs were relatively young (8 months to 5 years), and often presented for workup following sudden death, with no provided history of heartworm preventative administration. Heartworm (*Dirofilaria immitis*) is transmitted by mosquitoes, which take up larval microfilariae from the blood of infected animals, and transmit them to new hosts. Microfilariae develop into adult heartworms in the pulmonary arteries, resulting in endarteritis. Dogs can appear asymptomatic, but advanced infections and high parasite burdens result in pulmonary hypertension, the presence of heartworms in the right ventricle, and signs of right-sided heart failure. There are also sporadic reports of aberrant migration of heartworms or ascarids into the brain and other organs, with resultant localized inflammation and thromboembolic complications.

While treatable, heartworm is potentially fatal, and prevention is the preferred approach to combating heartworm infection. Veterinary practitioners can provide appropriate protocols for preventative parasite medication and annual antigen detection testing. *AHL*

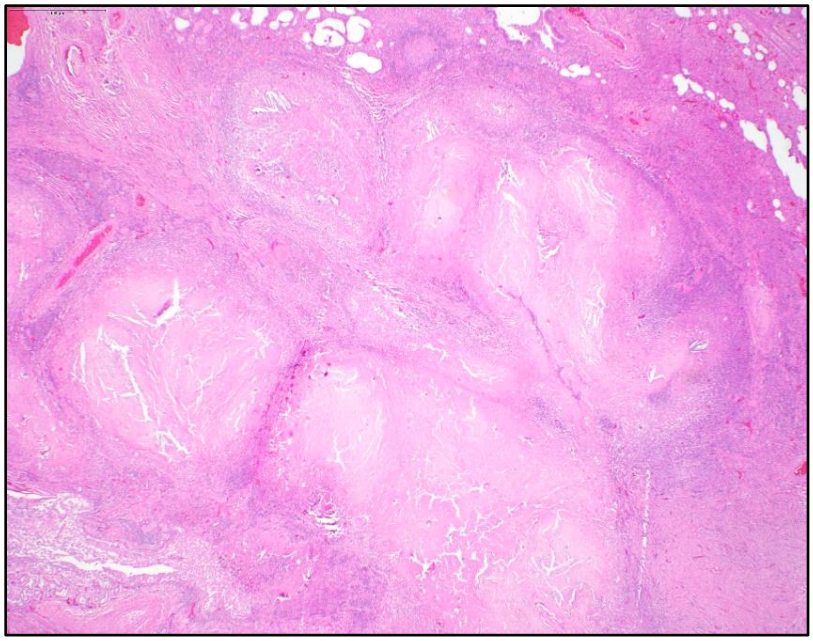

**Figure 1.** Pulmonary endarteritis, periarterial granulomatous inflammation secondary to infection with heartworm. H&E stain.

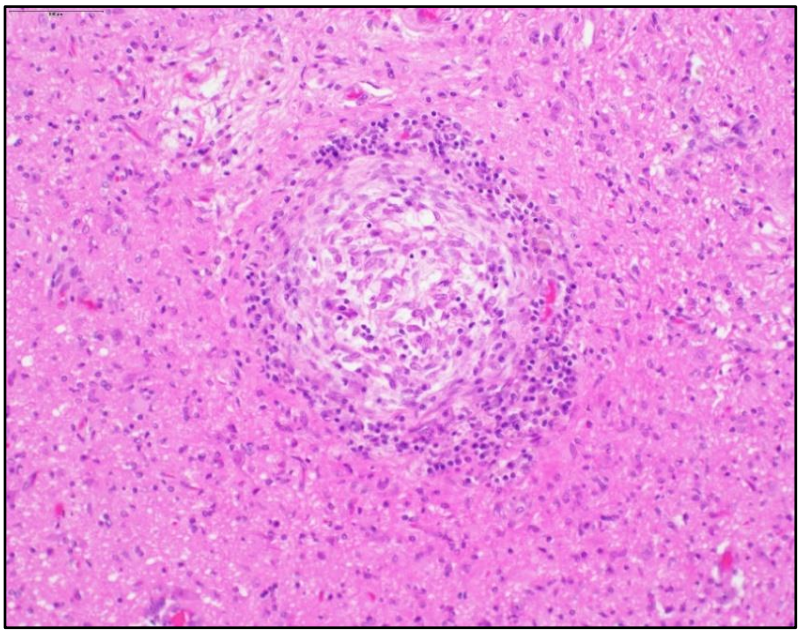

**Figure 2.** Focal granuloma in the midbrain attributed to aberrant parasite migration. H&E stain.

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# Lung fluke (*Paragonimus kellicotti*) infection in dogs and cats

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AHL Newsletter 2022;26(2):26.

In late 2021, several cases of lung fluke infection in dogs from the eastern shore of Lake Huron, ON area were reported to the OAHN Companion Animal Network. In North America, the most common lung fluke affecting dogs and cats is the trematode *Paragonimus kellicotti*. This species is found throughout the Mississippi and Great Lakes waterways. Several other *Paragonimus* species endemic to China, Southeast Asia, Japan, Central and South America can also infect pets. A search of case records from the AHL database (2007 – 2022) revealed 12 positive fecal samples for *Paragonimus* eggs, 3 from cats, 9 from dogs. In addition, there were 2 pathology cases in dogs with spontaneous pneumothorax, with the fluke/eggs visualized in lung biopsy samples (**Fig. 1**).

The natural definitive host of *P. kellicotti* is wild mink. Infected mink pass fluke eggs in their feces. Once in an aquatic environment, the parasite follows a complex life cycle involving aquatic snails as the first intermediate host, and freshwater crayfish as the second intermediate host. Immature forms can also survive free in water for days. Mink, as well as other animals including cats, dogs, and very rarely, people, can become infected by eating raw (or undercooked) crayfish, or eating other small animals (e.g., rodents) that feed on crayfish and act as paratenic hosts. Pets cannot spread the parasite directly to humans. Once ingested, the immature flukes are released and migrate from the intestinal tract through the body cavities until they reach the lungs, where they mature into adults and form cysts in the bronchioles after 2-3 weeks. Fluke eggs are coughed up, swallowed and passed in the feces. The prepatent period in cats is approximately 5-7 weeks.

Clinical signs of infection can range from none, or a mild cough, to varying degrees of dyspnea. In severe cases, animals may develop bronchiectasis, hemoptysis, or spontaneous pneumothorax. Detection of *P. kellicotti* eggs in the feces typically requires fecal sedimentation, as sensitivity of routine fecal flotation is poor (note: fecal centrifugal sedimentation can be specifically requested at AHL Parasitology, test short code *fcsed*). Eggs can also be detected on transtracheal wash in some cases. Eggs have a brown shell with a distinct operculum on one end, and are approximately 75-118 um x 42-67 um in size (**Fig.1**). Cysts in the lungs may be noted on thoracic radiographs or CT, and can be an incidental finding in subclinical cases. The flukes have a predilection for the caudal lobes, particularly the right lung. Peripheral eosinophilia may also be present.

There are no drugs available in Canada or the US with a label claim for treatment of *P. kellicotti* in dogs or cats; however, praziquantel and fenbendazole are reported to be effective. Treatment with either drug may need to be repeated in some cases to fully eliminate the infection. Treatment with praziquantel PO in cats can be problematic due to the bitter taste of some formulations, and may be cost-prohibitive in large dogs. Treatment is recommended even in subclinical cases due to the risk of the infection leading to acute pneumothorax. Uncomplicated infections generally respond well to antiparasitic treatment. In severe cases, lung lobectomy may be required.

A veterinary infosheet on *P. kellicotti* is available from the OAHN Companion Animal Network
https://www.oahn.ca/resources/paragonimus-kellicotti/

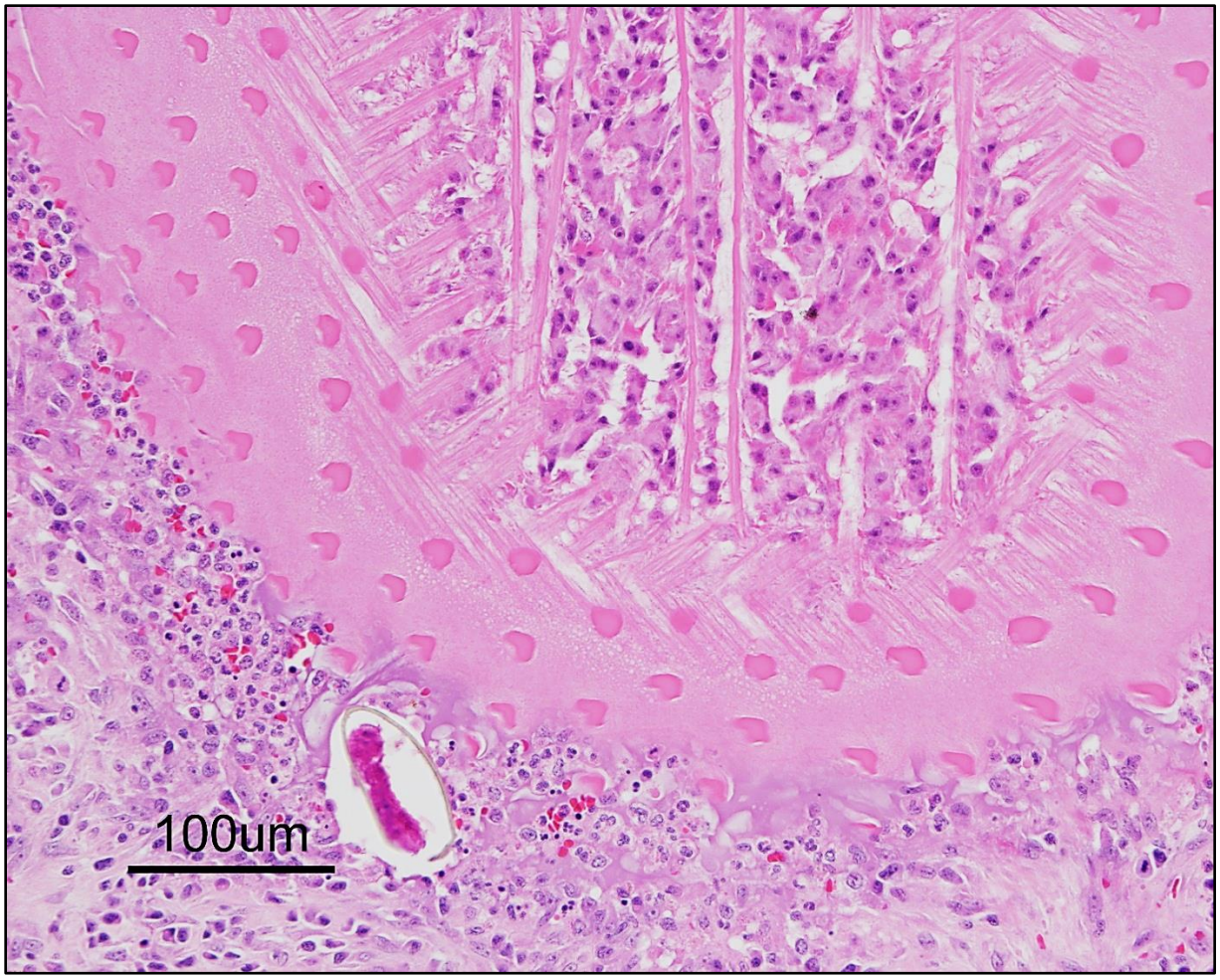


**Figure 1.** Histologic view of part of an adult fluke within the bronchiole of an infected dog, showing the thick outer eosinophilic tegument with short spines, and subtegmental musculature. A partially-ruptured, yellow-brown operculate egg is free within the adjacent inflammatory cell debris within the lumen of the bronchiole. This dog developed spontaneous pneumothorax, and a lung lobectomy was performed and submitted for histology. H&E stain. *AHL*

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