Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

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Update from the Director

The view from the Director's office

The restrictions imposed by the second wave of COVID-19 are starting to ease slightly in the Guelph area. However, we appear to be entering a very uncertain stage of the pandemic due to the increased number of cases caused by the highly contagious variants of concern. At AHL, we continue to monitor public health guidelines and to augment workplace protective measures for our staff who are performing an essential service for veterinarians, livestock producers and animal owners throughout Ontario. We expect that you are taking similar actions in your clinics and operational facilities.

It seems appropriate therefore that our "Staff highlights" article this month focuses on occupational health and safety in the veterinary workplace. Dr. Margaret Stalker is a pathologist at the Guelph AHL laboratory and she is also the co-chair of the Local Joint Health and Safety Committee in PAHL Building 89. Her advocacy of safety precautions and skill in interpreting sometimes complex requirements are very valued assets to AHL operations. Her expertise was also recognized by the OVMA which asked her to contribute to its health and safety guidelines.

Let's all hope that successful deployment of vaccines will begin to bring this pandemic under control. Until then, best wishes for continued health and safety.

Maria Spinato, Director

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

AHL Guelph Specimen Reception update

Jim Fairles, Jennifer Zoethout

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AHL Newsletter 2021;25(1):3.

1. **Courier update**: Although Purolator courier service has returned to a somewhat normal delivery schedule, there are still times when shipments are delayed. This has become noticeable again during the lockdown and around special dates (where general courier business is increased). These are beyond AHL's control and unfortunately, all of the large courier companies are experiencing the same issues. Saturday delivery (extra costs are incurred) remains unpredictable, is only available in certain areas, and is not recommended. AHL does have some local courier options (additional costs are involved). Please feel free to contact us if you would like to discuss courier options.

2. **Packaging submissions**: Due to courier delays and especially with warmer weather approaching, please ensure you are packaging submissions accordingly. Our submission instructions and overview videos are available to watch on our website. Any major announcements are also available there (inclement weather, holiday hours etc.): https://www.uoguelph.ca/ahl/

3. **Testing options at AHL**: We continually keep apprised of new and innovative testing options at AHL. Our submission forms have our main and most frequent testing options. If there is a test that you are looking for and do not see, please inquire. We are part of a large network of Canadian and American labs and can send samples throughout these countries for specialized testing, provided all permits are in place. We also constantly have new and updated testing methods in development. We are always interested in feedback from our clients around new tests that have potential for improving the health of the animal population. AHL continues to add combinations/panels of tests as well. Listed below are some of the new tests introduced at AHL in 2020. Details pertaining to specimen type, volume, turnaround time etc. can be found in our fee schedule online: https://www.uoguelph.ca/ahl/tests
   For the veterinary community, please email ahlinfo@uoguelph.ca for a user name and password for pricing if you do not already have access. Pricing information is not available to the general public.

   a. *Anaplasma marginale* and *A. centrale* - duplex qPCR (anapcr)
   b. Avian metapneumovirus (AMPV) – ELISA (ampve)
   c. Comprehensive Bovine Respiratory Disease Panel (brsppnl): Coronavirus PCR, Bovine respiratory virus panel - PCR (BoHV-1/IBR, BPIV-3, BRSV), BVDV Type 1 and 2 PCR, Bacterial culture.
   d. *Cryptosporidium* species – PCR (crypto)
   e. Swine dystrophin – genotyping (dystype)

AHL

Selected zoonotic pathogens and diseases from Ontario identified at the AHL, 2020

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AHL Newsletter 2021;25(1):4.

Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens in many animal specimens annually (Tables 1 and 2). The percentage of animals identified as positive for leptospirosis was relatively unchanged in 2020 in all species. The total number of submissions tested was about the same as 2019 or decreased slightly, except for dogs in which the number tested was down from to 214 to 171. These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates. They do not take into account vaccination status, as all species except horses may be routinely vaccinated for leptospirosis. Monitoring programs are not included. *Brucella canis* results are positive on the 2ME-RSAT. *AHL*

Table 1. Number of cases with selected zoonotic pathogens isolated and/or identified at the AHL, 2020.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2020	2019
Ascarids (incl *T. canis, T. cati, T. leonina, Baylisascaris* sp.)		4	7			46	1	25	6	4	**93**	**75**
Blastomyces dermatitidis								7			**7**	**5**
Bordetella bronchiseptica	3	35			1		1	2	3	6	**51**	**62**
Borrelia burgdorferi (Lyme disease), serology			25					6	1	2	**34**	**27**
Brucella sp. *(non-abortus)*								14			**14**	**79**
Campylobacter coli/ jejuni/ fetus subsp. *fetus*	1			5		1					**7**	**17**
Chlamydia sp.		1		9	9						**19**	**9**
Clostridium difficile			1					1		1	**3**	**5**
Coxiella burnetii (Q fever)	6			22	17						**45**	**62**
Cryptococcus sp.											**0**	**1**
Cryptosporidium sp.	218			6	5					10	**239**	**252**
Eastern equine encephalitis virus			4								**4**	**7**
Echinococcus multilocularis								2			**2**	**2**
Giardia sp.	2				1			32			**35**	**38**
Listeria monocytogenes	11			9	5						**25**	**29**

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2020	2019
Methicillin-resistant *Staph aureus* (MRSA)		3				1		1		1	**6**	**4**
Methicillin-resistant *S. pseudintermedius* (MRSP)								56	5	1	**62**	**48**
Rabies virus										5	**5**	**9**
Salmonella enterica	56	78	4	4		29	54	2		18	**245**	**264**
Streptococcus suis	2	144				2					**148**	**140**
Streptococcus equisimilis	2	31	13					2			**48**	**71**
Streptococcus zooepidemicus	2	1	133	1				3			**140**	**177**
Toxoplasma sp.				10	4					1	**15**	**10**
Verotoxigenic *E.coli (*VTEC)	5	1									**6**	**11**
West Nile virus			1							26	**27**	**25**
Yersinia enterocolitica	3	1						1			**5**	**6**
Total											**1285**	**1435**

Table 2. *Leptospira* spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL, 2020

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Other
L. autumnalis	13	4	32	67	
L. bratislava	42	3	34	58	1
L. canicola	16	3	11	38	
L. grippotyphosa	7	2	4	37	
L. hardjo	29	3	11	17	1
L. icterohaemorrhagiae	29	3	17	65	
L. pomona	31	3	13	41	
IHC or PCR-positive	3	0	0	6	
Positive/tested cases	**56/192**	**4/31**	**38/61**	**104/171**	**1/3**
% positive	29.17%	12.90%	62.30%	59.06%	57.14%
% positive, 2020/2019	29/29%	13/15%	62/63%	59/53%	57/24%

OAHN Update – March 2021

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AHL Newsletter 2021;25(1):6.

The Ontario Animal Health Network has been busy throughout the fall and winter, releasing many new veterinary, producer, and owner reports, as well info sheets, infographics, resources, a podcast series, and much more.

New OAHN Resources

Infectious Laryngotracheitis (ILT) Biosecurity Infographic and Info Sheet
The OAHN Poultry Network has created new resources to help producers with ILT in their flocks. The ILT biosecurity infographic and info sheet cover signs of ILT, how it is spread, how to protect your flock, and what to do if you have sick birds.

Humane Slaughter for Small to Medium Size Aquaculture Producers Info Sheet and Case Study
The OAHN Fish Network created an info sheet and case study that cover the materials and methodology, and everything else you need to know about the humane slaughter for aquaculture producers.

Managing the Risk of Malignant Catarrhal Fever (MCF) from Sheep to Bison
The OAHN Small Ruminant Network prepared an info sheet for small ruminant and bison producers on how to manage the risk of malignant catarrhal fever from sheep to bison.

Antimicrobial Stewardship Resources
The OAHN Companion Animal Network has created an antimicrobial stewardship resources page, which collects Veterinary antimicrobial stewardship resources, antimicrobial use guidelines, additional veterinary resources and statements, and related public health resources.

Ontario Animal Health Network (OAHN) Companion Animal Expert Network Public Health Report 2020
Check out the Companion Animal Network's 2020 public health report, which contains public health information for all networks, including *Salmonella* Dublin septicemia in a puppy, Brucella in breeding kennels: Project summary, *Echinococcus multilocularis*, Backyard bird resources, and much more.

OAHN COVID-19 Mini-Podcasts
The OAHN mini-podcast series on COVID-19 precautions in veterinary clinics features quick 3-5 minute "lighting rounds" with advice and tips from infection control expert and University of Guelph professor Dr. Scott Weese. Check out all of the podcast here: http://oahn.podbean.com/category/covid19/

New Reports and Resources
The latest network reports for companion animals, bovine, swine, poultry, and equine have been posted to the OAHN site under "Network Reports".

We have lots of other **new reports, lab data, and resources**. Be sure to check out **OAHN.ca**

Staff highlights – Health and safety in the veterinary workplace

Margaret Stalker

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AHL Newsletter 2021;25(1):7.

Recent health and safety protocols in all our workplaces have necessarily been tightly focused on reducing the risk of exposure to and transmission of COVID-19. However, this is also a good time to reflect and remember that these measures are part of the larger picture of occupational health and safety in the veterinary workplace.

Health and safety regulations and practices are designed to protect the well-being of all workers, including veterinary practice owners and managers, veterinarians, veterinary technicians, hospital staff, as well as clients and patients. These practices form an important part of everyday work life. The Occupational Health and Safety Act and the Workplace Safety and Insurance Act set out the rights and responsibilities of workplace employees and employers in Ontario workplaces, and workplaces are bound by law to follow the regulations outlined by these acts, as applicable. Other acts such as the Environmental Protection Act also regulate some activities in veterinary practices.

Some examples of these responsibilities include:

- Providing and documenting training of all clinic workers in basic occupational health and safety awareness, including information about workplace-specific hazards and providing WHMIS training. In the veterinary practice context, such workplace hazards may include potential exposure to infectious microorganisms, hazardous chemicals and pharmaceuticals, compressed gases, physical and ergonomic risks involved in animal handling and restraint, exposure to ionizing radiation, and hazards from various specialized equipment and sharps.
- Providing first aid equipment, facilities and trained personnel.
- Assigning a health and safety representative (in workplaces with between 5-19 workers) or creating a Joint Health and Safety Committee of at least one worker and one management member (in workplaces with 20 or more workers).
- In workplaces with a Joint Health and Safety Committee, ensuring proper certification training of at least one worker and one management representative. The primary role of the representative/committee is to regularly inspect the workplace, identify potential health and safety issues and bring them to the attention of the employer. Committees with certified members are required to meet every three months to discuss health and safety in the workplace.
- Providing a clinic health and safety bulletin board, displaying at a minimum:
    - a copy of the Ontario Health and Safety Act.
    - a copy of the Ontario Ministry of Labour (MOL) poster, “Health & Safety at Work: Prevention Starts Here”.
    - a list identifying the health and safety representative/committee members
    - in workplaces with more than 5 regularly employed workers, the workplace health and safety policy, workplace violence policy and workplace harassment policy. More details and examples of these are available on the MOL website.
    - a copy of the Joint Health and Safety Committee meeting minutes.
- Ensuring proper hazardous waste registration and disposal.

- Registration of radiology equipment with the Ontario Ministry of Labour, training of workers in radiation safety, and training a designated staff member as the clinic radiation safety officer.

While this list is not exhaustive, several excellent resources are available online, including the online Health and Safety portion of the OVMA website, the Ontario Ministry of Labour website, and the WSIB.

Here's to making safe practice a long-term priority! *AHL*

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1. OVMA website: Health and Safety https://www.ovma.org/veterinarians/health-and-safety/
2. Ontario Ministry of Labour, Training and Skills Development https://www.labour.gov.on.ca/english/hs/index.php
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4. Deimling B, Safety is no accident. OVMA Focus Magazine, May/June 2019, pp 22-23.

RUMINANTS

Brainstem *Parelaphostrongylus tenuis* in a domestic sheep

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AHL Newsletter 2021;25(1):8.

A 2.8-year-old, intact female Katahdin sheep presented to the Large Animal Clinic of the Ontario Veterinary College's (OVC) Health Sciences Center with a several day history of ataxia and eventual recumbency. Initial clinical signs were reported as repeated falling to the left. The sheep was treated with oxytetracycline and thiamine with minimal improvement, and the condition progressed to lateral recumbency. Three other sheep on the farm showed similar clinical signs, one of which died prior to treatment, while two others were treated with thiamine, oxytetracycline, and multivitamins and recovered. All sheep on the farm were on a forage-only diet. Though on similar feed and housing, this animal was not in direct contact with the other affected sheep.

On presentation to OVC, the sheep was dull and obtuse but responsive to external stimuli with an elevated respiratory rate (48 breaths/minute), tacky and pale pink mucous membranes, a slightly delayed capillary refill time (~2 seconds), struggled to lift herself into sternal recumbency and had multiple cranial nerve deficits. In hospital, she repeatedly struggled with an increased respiratory rate with lung consolidation present on thoracic ultrasound which was attributed to the lateral recumbency. On the second day of hospitalization, signs consistent with a seizure began, and they occurred multiple times while in hospital. She was treated with intravenous fluids, oxytetracycline, systemic anti-inflammatories, gabapentin, diazepam, thiamine, gastroprotectants, and oxygen therapy. Approximately two days after presentation, humane euthanasia was elected due to poor prognosis.

On necropsy, there was a large amount of foam in the trachea, and the lungs were heavy and wet with some separation of the septa by edema. On the left ventral side of the caudal brainstem was an approximately 1.5 cm diameter area of hemorrhage. No other gross findings were appreciated on necropsy. Histologically, within the cerebellar peduncle of the brain, there was a focal area characterized

by perivascular cuffs of eosinophils (confirmed by Luna stain), lymphocytes, and plasma cells. The neuroparenchyma in this area was vacuolated with swollen axons and aggregates of foamy macrophages. On further sectioning of the brainstem to target the area of focal hemorrhage, multiple profiles (cross and longitudinal sections) of a nematode were present focally replacing the neuroparenchyma in the cerebellar peduncle (**Fig. 1**). The parasite had a body cavity enclosed within a thin cuticle with accessory hypodermal cords and polymyarian-coelomyarian musculature. The intestine was large and composed of a few multinucleated cells (**Fig. 1 inset**) and there was also a reproductive tract. These features are consistent with *Parelaphostrongylus* sp., suspected to be *P. tenuis* based on the location.

To confirm the identity of the parasite, DNA sequencing was pursued. The formalin-fixed paraffin-embedded block was manually cored using a 0.6 mm punch to obtain the parasite with minimal surrounding ovine brain tissue. Initially, universal ribosomal internal transcribed spacer (ITS) region primers produced a positive band of the correct size by gel electrophoresis, but there was insufficient concentration to allow sequencing. *Parelaphostrongylus*-specific primers, as previously described (1,2) were used to obtain a sufficient product that was sent for sequencing. There was 100% sequence similarity (156/156 bp) to a partial segment of the ITS2 (second internal transcribed spaces of the ribosomal RNA gene) region of both *P. tenuis* and *P. andersoni*. This supports an etiologic diagnosis of *P. tenuis* since the brain, rather than muscle was affected (as would be expected with *P. andersoni*), and explained the clinical signs demonstrated by this sheep. It is unknown whether the other sheep were also affected by this parasite, as no follow-up testing was pursued in these animals.

P. tenuis most commonly infects white-tailed deer (*Odocoileus virginianus*) and generally produces no clinical signs in this species. The nematodes complete their life cycle and generate first stage larvae that are expelled in the feces. These larvae enter gastropods (slugs, snails), their intermediate hosts, where they develop into infective third stage larvae. The intermediate host is then ingested by either white-tailed deer, where they complete their cycle, or abnormal hosts, where clinical disease is most commonly seen. Following ingestion, the larvae exit the stomach and migrate aberrantly, most commonly into the spinal cord or less frequently, into the brain (3). These aberrant migrations have been reported in a variety of species, including sheep, with camelids reported to be most susceptible and cattle most resistant (3). Postmortem diagnosis can be difficult as the parasite is not commonly found in histologic sections and therefore, it is often presumptive based on clinical signs, cerebrospinal fluid analyses, response to therapy, and/or characteristic focal inflammatory lesions in the brain (2,3). *AHL*

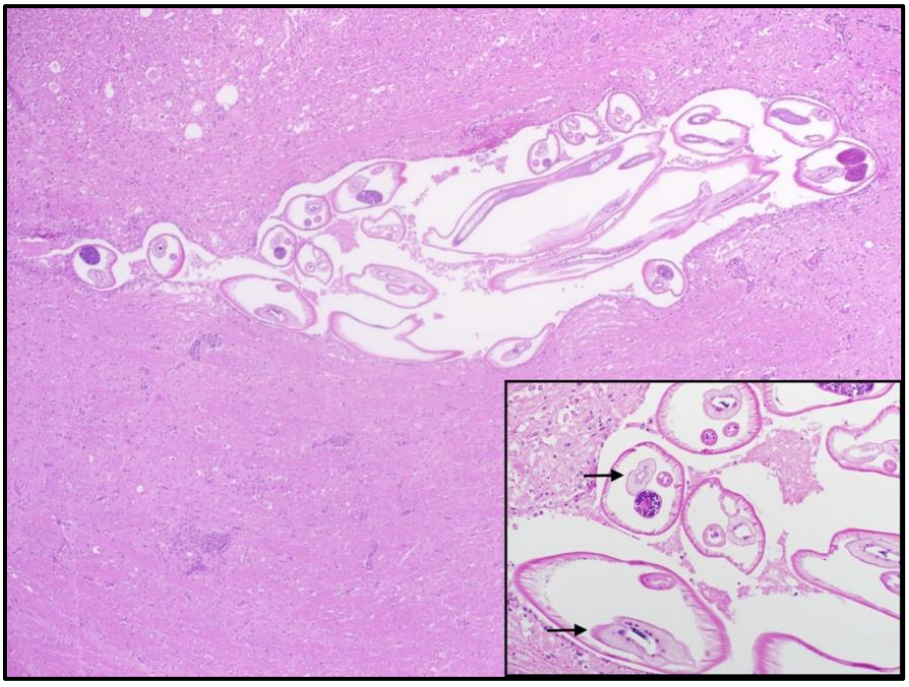

Figure 1. Replacing the neuroparenchyma are multiple cross and longitudinal sections of a nematode parasite consistent with *Parelaphostrongylus tenuis* (inset: higher magnification of the parasite, arrows indicating the multinucleated intestine). (H&E)

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2. Mitchell KJ et al. Diagnosis of *Parelaphostrongylus* spp. infection as a cause of meningomyelitis in calves. J Vet Diagn Invest 2011;23(6):1097-1103.
3. Nagy DW. *Parelaphostrongylus tenuis* and other parasitic diseases of the ruminant nervous system. Vet Clin Food Anim 2004;20:393-412.

Ovine abortion with fetal congenital anomalies – Cache Valley virus (CVV)

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AHL Newsletter 2021;25(1):10.

An aborted mixed-breed sheep fetus from a flock of 175 commercial ewes in the Durham region was submitted for postmortem examination. This near-term fetus exhibited several somewhat subtle skeletal anomalies, including: inferior brachygnathia with an abnormally thickened and flattened skull; scoliosis and flexural limb deformities; as well as brain and spinal cord malformation and hypoplastic hind limb musculature (**Fig. 1**). There was also a mild placentitis evident on histologic examination. RT-PCR testing at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) was positive for Cache Valley virus (CVV). Other additional tests were negative for significant pathogens. The flock, which lambs all year round, experienced seven abortions with similar fetal anomalies during the month of December 2020. A wave of similar abortions had also occurred in December of 2019.

We periodically see small clusters of ovine abortion cases associated with CVV infection submitted to the AHL, often on a 3-5 year interval. CVV is a mosquito-borne, potentially zoonotic Orthobunyavirus considered to be endemic in Ontario. Although rarely diagnosed, infection in humans can cause fever, headache, meningitis or encephalitis. In veterinary species, CVV infection can cause infertility, abortion, stillbirths and congenital anomalies in sheep and goats. Fetal malformations in sheep are associated with infection of the ewe at 28-48 days of gestation. The population of infected mosquitos reaches its height during late summer/early fall, therefore, abortions/stillbirths typically occur during December/January. The TVMDL offers both PCR testing (placenta, fetal brain) and virus neutralization (VN) assay (fetal thoracic cavity fluid, serum samples from ewes). This case was unusual in that the PCR testing was positive. The virus is typically cleared by the time the fetus is aborted, and fetal VN testing is often more reliable. Titres in the ewes confirm exposure, and animals exposed to the virus may have lifelong immunity. *AHL*

Figure 1. CVV positive aborted ovine fetus with inferior brachygnathia, an abnormally thickened and flattened skull, scoliosis and flexural limb deformities.

SWINE

Streptococcus equi subsp. *zooepidemicus* septicemia: First confirmed case in Ontario swine

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AHL Newsletter 2021;25(1):11.

A diagnostic workup was carried out in a swine herd to investigate sow anorexia, unexpected deaths (5 times normal rate), and an increase in the rate of abortions / pregnancy loss (range of 2-4 times normal over 15 weeks). Postmortem examination of 1 dead sow identified fibrinous peritonitis and congested, edematous lungs. Histological lesions included: pulmonary microvascular thrombosis with alveolar edema and fibrin exudation; fibrinosuppurative perisplenitis (peritonitis); and splenic congestion with intralesional bacterial cocci. The histologic findings supported septicemia as the cause of the sow's death. *Streptococcus equi* subsp. *zooepidemicus* was isolated by bacterial culture in low to moderate numbers from each of spleen, lung, and kidney. PRRSV was not detected in lung by PCR. Surveillance PCR testing for African swine fever was carried out through the CanSpot ASF project, and the result was negative. Abortion cases were not investigated, but were presumed to be the result of maternal illness.

Recent literature reports have described *S. zooepidemicus* septicemia as the cause of unexpected deaths and abortions in sow herds in Manitoba, and in mature animals and feeder pigs at an assembly yard and an abattoir in the US (1,2). In the US cases, mortality was significant at 30-50% over a period of 8-10 days (2).

Postmortem lesions reported are those of septicemia and are not specific to this organism, but may include:

- mild mucopurulent rhinitis
- pulmonary edema
- hemorrhagic lymphadenopathy (particularly submandibular, cervical, and bronchial nodes)
- splenomegaly (mild to moderate)
- gall bladder edema
- fibrinous epicarditis
- fibrinous perisplenitis
- multiorgan congestion

Isolation of the organism from filtering organs (spleen, lung, kidney) is required for diagnosis.

Advanced sequencing techniques have identified a common strain of *S. zooepidemicus* isolated from the reported cases that is thought to be more virulent than typical commensal strains found in pigs and other species. For the *S. zooepidemicus* isolate from the Ontario case, whole genome sequencing (WGS) and multilocus sequence typing (MLST) at the Animal Health Laboratory identified a sequence type (ST194) similar to isolates from the recent Manitoba and US outbreaks.

S. zooepidemicus is a potential zoonotic pathogen, similar to *S. suis*. Zoonotic transmission has not been reported from any of the recent North American outbreaks. *AHL*

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PRRSV oral fluids ELISA testing at the AHL

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AHL Newsletter 2021;25(1):12.

Oral fluid samples are non-invasive to pigs, simple and cost-effective to collect. These characteristics have made them attractive as samples for detecting antibodies against porcine reproductive and respiratory syndrome virus (PRRSV). According to manufacturer's data, the IDEXX PRRSV oral fluid ELISA has a diagnostic sensitivity of 100% and a diagnostic specificity of 98.7% when the manufacturer's cut-off of sample to positive (S/P) ratio ≥ 0.4 is used. Because the test specificity is less than 100%, it will occasionally generate false-positive results. It has also been reported that non-specific positive oral fluid ELISA results can be generated in PRRSV-negative pigs that were fed with a feed containing spray-dried porcine plasma (1).

Regardless of the cause, unexpected positive results have limited the application of oral fluid ELISA for PRRSV surveillance. A recent study suggested that increasing the manufacturer's ELISA cut-off to S/P ratio ≥ 0.8 improved the specificity of the test and decreased the chance of false-positive reactivity in presumed-negative herds (2). In another study, raising the cut-off to S/P ratio of 1.0 increased the oral fluid ELISA specificity to 99.2%, but still did not completely remove false-positive reactivity. However, as a consequence of raising the cut-off, the test sensitivity decreased to 96.5% (3).

Therefore, results of IDEXX PRRSV oral fluid ELISA tests performed at AHL will continue to be reported using the manufacturer's cut-off of S/P ratio ≥ 0.4 which is appropriate for diagnostic applications and ensures the optimal test sensitivity. While we have considered a different cut-off for surveillance applications, our accredited status does not permit us to alter the current kit recommendations. We have reached out to the manufacturer who is aware of the situation and is investigating possible options to increase the utility of the test for both diagnostic and surveillance purposes.

When performing the oral fluids PRRSV ELISA in presumed-negative herds, practitioners may use their discretion to consider a cut-off of S/P ratio ≥ 1.0 to increase the test specificity for surveillance purposes. To support this usage, the following comment has been added to all AHL PRRSV oral fluid ELISA test reports:

"Test results are interpreted using the manufacturer's cut-off which is appropriate for diagnostic applications:
Negative: S/P ratio less than 0.400
POSITIVE: S/P ratio equal to, or greater than 0.400

However, S/P ratio cut-off equal to, or greater than 1.000 may be suitable to increase the test specificity for surveillance in presumed-negative herds (Henao-Diaz A, et al. Prev Vet Med. 2021. Adapting a PRRSV oral fluid antibody ELISA to routine surveillance).
Unexpected positive results should be further investigated by additional testing with PRRSV serum ELISA and/or PRRSV PCR." *AHL*

References

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AVIAN/FUR/EXOTIC

Poultry lameness: Examination and sample collection

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AHL Newsletter 2021;25(1):13.

Veterinarians who treat commercial or backyard flocks will inevitably encounter lameness issues. The source of the lameness can exist anywhere from the spine to the foot, therefore, postmortem examination must include a thorough examination of the entire neuro-musculo-skeletal system. It is also important to submit appropriate samples to the lab for follow-up testing. The following is a description of the postmortem examination process and photographs of potential samples to collect.

Examination should start at the foot and work up towards the spine.
1) Foot – Should be examined for dermatitis. The American Association of Avian Pathologists (AAAP) has a reference document for footpad scoring that you can access here: https://aaap.memberclicks.net/assets/documents/store/broiler%20paw%20scoring%20guide%20aaap%202015%20final.pdf

2) Hock – Examine the skin behind the hock for swelling or discolouration around the gastrocnemius tendon. Pluck the feathers around the joint to ensure the feathers will not contaminate any sampling attempts. Cut the hock open from the front, being careful not to contaminate internal tissues or fluid (**Fig. 1**). Swab using gel swabs for bacterial culture or viral transport media (VTM) swabs for PCR testing (**Fig. 2**).

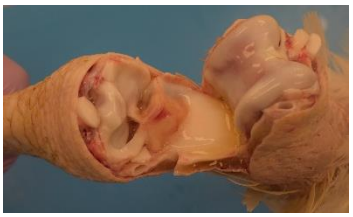


Figure 1. Opened hock

Figure 2. Hock swab (gel)

3) Tendon (Gastrocnemius) – Once the hock joint is opened and swabbing is complete, continue cutting up the sides of the tendon behind the hock. Continue opening until you can access the gastrocnemius tendon to make a high transverse cut (**Figs. 3,4**). You can then make another transverse cut nearer to the joint to collect a short length of tendon to submit for histology (**Fig. 5**). On histology, a cross section through this tendon allows the pathologist to evaluate multiple tendons. In commercial turkeys where turkey arthritis reovirus (TARV) is suspected, it is important to also collect the group of tendons running along the back of the shank for histopathology (**Fig. 6**). Including the skin keeps the tendons attached for ease of trimming.

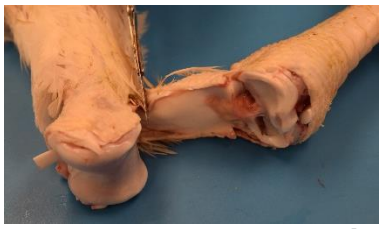


Figure 3. Gastrocnemius tendon

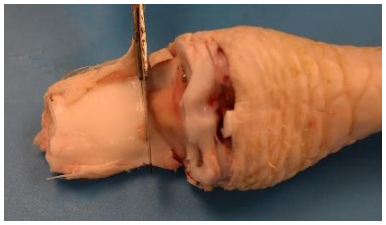


Figure 4. Transverse section

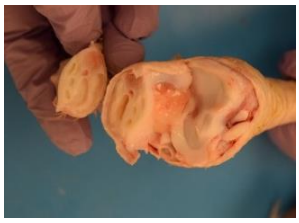


Figure 5. Submit piece of tendon.

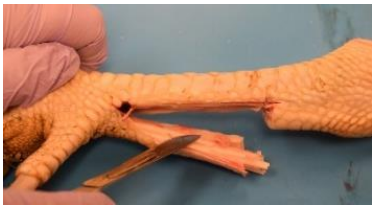
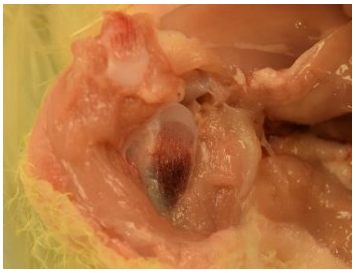


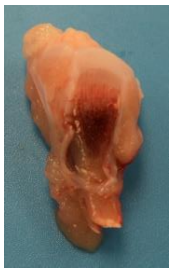
Figure 6. Shank tendons.

4) Stifle (Knee) – This can be a challenging joint to open. If the bird(s) are well fleshed there will be a line of fat running at a downward angle toward the body. Cutting along this line of fat will open this joint (**Fig. 7**). Look for increased amount of discoloured fluid.

Figure 7. Stifle

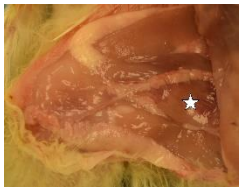
5) Tibiotarsal bone (proximal) – In younger birds, the growth plates should be examined for abnormal development (rickets, tibial dyschondroplasia (TD)) or localization of bacteria (osteomyelitis). For histology, it is important to consistently collect the same sample for identification of TD lesions, and to provide comparable samples among birds for examination. Along the medial surface of the proximal tibiotarsal bone, slice the medial surface of the bone to reveal the growth plate without cutting the growth plate or articular cartilage off (**Fig. 8**).
If abnormalities are suspected, collect the proximal end of the tibiotarsal bone, trim most of the musculature off, and place into formalin (**Fig. 9**).

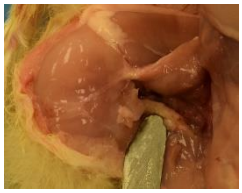




Figures 8, 9. Growth plate, remove entire proximal tibiotarsal bone and formalin fix.

6) Skeletal muscle and sciatic nerve – If Marek's disease is on your list of rule-outs, you will need to submit sciatic nerve for histologic evaluation. Along the medial aspect of the leg, there is a triangular muscle (pubo-ischio femoralis) that is a key muscle to collect and submit if you are suspecting ionophore toxicity (**Fig. 10**). This is also the muscle to reflect to locate the sciatic nerve (**Fig. 11**).





Figures 10, 11. Reflect muscle (white star) to collect sciatic nerve.

7) Femoral head – When the leg is initially reflected, the leg can be forced laterally to pop the femoral head out of the acetabulum (so the bird will lie flat) (**Fig. 12**). If the femur fractures adjacent to this joint (neck of the femur), this is called femoral head necrosis (or bacterial chondronecrosis with osteomyelitis) and can indicate the bone is weakened from a bacterial infection. The bone marrow can be swabbed (gel swab) at this point to submit for bacterial culture. Examine the femoral head and acetabulum for any abnormalities.

Figure 12. Exposed femoral head.

8) Spine – The final location to examine is the spinal column. There is one movable joint in the spine of poultry and it is located in the region of the caudal lungs/cranial kidneys. Depending on the size of the bird, take either a scalpel or knife and split the spine right down the middle (**Fig. 13**). Pry the spine open and look for irregularities in the formation of the spine (kinky back) or abscess formation in the vertebrae (swab for bacterial culture) (**Fig. 14**).

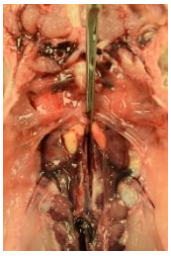


Figure 13. Split spine.

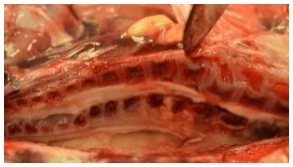


Figure 14. Examine spine for abscesses.

Additional sampling notes: The sciatic nerve also runs through the kidney. If there are any kidney lesions, it could be impinging on the nerve and causing lameness. *AHL*

HORSES

Equine granulocytic anaplasmosis

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AHL Newsletter 2021;25(1):15.

A 16-year-old quarter horse gelding presented to the referring veterinarian with a 5 day history of lethargy, inappetence, and weakness. Pyrexia was identified during physical examination, feces were normal, and no proprioceptive deficits were identified. Samples for a comprehensive CBC, equine biochemistry profile, fecal flotation, and equine herpes virus 1 PCR were submitted to the Animal Health Laboratory. Herpes virus PCR was negative and fecal flotation revealed the presence of both strongyles and *Anoplocephala perfoliata.*

The CBC demonstrated a marked thrombocytopenia of 38 $x10^9$/L. A moderate lymphopenia was identified, however, remaining leukocyte and erythrocyte values were within reference intervals. Review of the peripheral blood smear demonstrated the presence of one or more loose aggregates of blue-gray to dark blue coccoid to coccobacillary organisms within the cytoplasm of numerous neutrophils. These aggregates were consistent with morulae of *Anaplasma phagocytophilum* bacteria, the causative agent of equine granulocytic anaplasmosis (**Fig. 1**). A duplex quantitative PCR for both *A. phagocytophilum* and *Borrelia burgdorferi* was performed by the AHL molecular biology lab using EDTA blood; a strong positive result for *A. phagocytophilum* was obtained, and *B. burgdorferi* was negative.

Concurrent significant changes in the serum biochemistry profile included a marked increase in serum amyloid A (6431 mg/L), along with alterations in electrolyte concentrations. This patient responded well to antibiotic treatment. No follow-up laboratory data was available.

These laboratory findings were similar to those previously published (AHL Newsletter December 2015) which described *A. phagocytophilum* infection in an Appaloosa mare. Interestingly, both horses were from eastern Ontario. A 2017 OAHN (Ontario Animal Health Network) equine disease surveillance project evaluating the seroprevalence of *B. burgdorferi* and *A. phagocytophilum* in Ontario horses identified that 1% (5/551) of serum samples submitted from clinically healthy horses were positive for *A. phagocytophilum* antibodies utilizing a snap 4Dx ELISA test. Two of these horses originated from eastern Ontario, whereas the remaining three were distributed throughout the province.

A. phagocytophilum, formerly known as *Ehrlichia equi*, is the causative agent of equine granulocytic anaplasmosis (EGA). It is most likely transmitted in Ontario by the deer tick, *Ixodes scapularis*, which is also the tick that transmits *B. burgdorferi*, the causative agent of Lyme disease. EGA occurs most commonly in the late fall, winter, and spring. Horses are likely aberrant hosts, and do not appear to serve as reservoirs of *A. phagocytophilum*, as the presence of the organism is generally limited to the acute phase of disease. Naturally infected horses appear to have persistent humoral immunity for at least two years. The occurrence of subclinical infection in horses from endemic areas has been suggested.

EGA can have a variable presentation ranging from mild fever in young horses (<4 years of age), to more severe clinical signs including fever, limb edema, ataxia, jaundice and petechial hemorrhages secondary to vasculitis and thrombocytopenia. Body effusions and myopathies have occasionally been reported. Moderate to severe morbidity is occasionally seen with EGA, and occasional mortality has been reported. The disease is often self-limiting, and clinical signs usually last 7-14 days. Anemia, variable leukopenia and thrombocytopenia are usually identified with clinical cases.

The pathogenesis of EGA is poorly understood, however following inoculation by tick bites, the bacteria invade the hematopoietic and lymphoreticular systems. Peripheral sequestration, consumption, and destruction of peripheral blood components are all proposed as mechanisms of cytopenias.

Diagnosis relies upon clinical awareness of geographic areas for infection, consistent clinical signs, and supportive laboratory changes. Evaluation of a peripheral blood smear is imperative, although the number of granulocytes containing morulae may vary from 1% to 50% by day 3-5 of infection. If the horse is severely neutropenic, evaluation of a buffy coat may be helpful.

PCR can confirm the clinical diagnosis and may be particularly helpful in both early and late stages of the disease when morulae are difficult to detect. The AHL molecular biology lab offers a qPCR test for *A. phagocytophilum* (test code: lyPCR) which can be applied to whole blood, tissue, and ticks. *AHL*

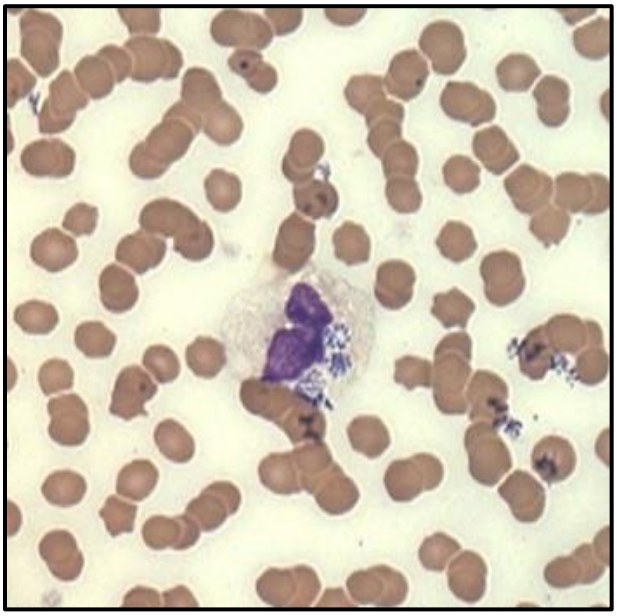

Figure 1. A neutrophil containing three *A. phagocytophilum* morulae.

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Alcohol and Gaming Commission of Ontario (AGCO) Equine Incidences in Ontario Racing program (EIOR): 2003 - 2020 postmortem summary

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AHL Newsletter 2021;25(1):17.

The Alcohol and Gaming Commission of Ontario (AGCO; formerly the Ontario Racing Commission, ORC) continues in its **proactive approach to advance racehorse welfare and safety of human and animal participants**. In 2003, Ontario became one of the first North American racing jurisdictions to require mandatory reporting of racehorse deaths in order to monitor, research and improve knowledge of why these events occur. Postmortem (PM) exams conducted at the Animal Health Laboratory (AHL) through the ORC Death Registry (DR, 2003-2016) and Equine Incidences in Ontario Racing (EIOR, 2016-current) programs continue to provide comprehensive data regarding the causes of morbidity and mortality in racehorses in this province. To date, PMs have been carried out on 1213 horses through these programs (**Table 1**). Annual variation in the number of PM cases reflects the discretionary requirement for PM of reported deaths on the part of the Registrar of AGCO.

A summary of significant PM findings is provided in **Table 2**. A comprehensive review of AGCO PM cases was recently conducted as part of a separate retrospective study (1). As a result of this study, some cases have been reclassified from data presented in previous editions of the AHL Newsletter.

Since 2015, **computed tomography (CT) of fractured and contralateral limbs** has been carried out on select DR and EIOR postmortem cases through collaboration with the Diagnostic Imaging section of the Ontario Veterinary College Health Sciences Center. The goal of this in-depth examination is to identify pre-existent lesions, primarily in bone, that contribute to catastrophic fractures. The protocol was continued in 2020, with CT imaging of all 19 limb fracture cases submitted for PM exam. **Pre-existent lesions in bone were identified by CT and considered potentially predisposing to fracture in 12 of 19 (63%) cases.**

Exercise-associated sudden death continues to be of special concern in the racing industry. At the AHL, a modified in-depth PM protocol is used in the evaluation these cases, with special emphasis on cardiovascular and respiratory systems. In 2020, the cause of death was investigated in 10 horses that died while exercising. Death was attributed to multiple causes including: significant pulmonary hemorrhage (3 horses); aortic rupture (1 horse); myocardial fibrosis and possible cardiac dysrhythmia (1 horse); and abdominal hemorrhage (2 horses; associated with pelvic fracture in 1 horse). No definitive cause of death was identified in 3 horses that died while exercising. Among all sudden death cases from 2003-2020, significant pulmonary hemorrhage was identified in 96/197 (49%) horses. The term 'equine exercise-associated fatal pulmonary hemorrhage' (EAFPH) is currently used to categorize these cases, in which extensive pulmonary hemorrhage and edema is identified postmortem. In 46/197 (23%) exercise-associated sudden death cases from 2003-2020, no potentially fatal lesions were identified, and the cause of death remained undetermined. It has been speculated that **exercise-associated cardiac dysrhythmia**, leading to acute heart failure and pulmonary hypertension, may be the underlying cause of death among many of these horses, and may also contribute to pulmonary hemorrhage in these animals (2). Typically, no morphologic lesions are detected in heart as a cause or result of fatal ventricular dysrhythmia, and the diagnosis cannot be confirmed based on PM findings. Studies of exercise-associated sudden death cases from other jurisdictions have not identified gross or histologic lesions in heart that are considered significant to the cause of death, based on comparison with control animals (3). In addition, no genetic contribution to the syndrome has been identified to date (3). *AHL*

Table 1. Breed distribution of AGCO EIOR submissions to the AHL, 2003-2020

Breed / year	Standardbred	Thoroughbred	Quarter Horse	Total
2003	63	59	0	**122**
2004	81	60	0	**141**
2005	59	51	0	**110**
2006	58	46	2	**106**
2007	66	53	3	**122**
2008	27	24	0	**51**
2009	28	16	1	**45**
2010	22	8	2	**32**
2011	24	18	4	**46**
2012	20	14	0	**34**
2013	19	26	2	**47**
2014	21	22	8	**51**
2015	29	24	3	**56**
2016	15	32	3	**50**
2017	26	34	2	**62**
2018	16	33	1	**50**
2019	12	35	0	**47**
2020	5	36	0	**41**
Total	**591**	**591**	**31**	**1213**

Table 2. Significant postmortem lesions identified in AGCO EIOR submissions to the AHL, by body system, 2003-2020.

Diagnoses by body system:	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
Fracture / limbs	51	69	48	43	58	16	3	7	5	2	22	23	25	27	34	29	27	19
Fracture / other	10	4	6	11	8	5	0	3	6	2	2	8	4	4	1	1	4	4
Non-fracture musculoskeletal	8	7	8	7	5	4	4	2	0	0	2	3	4	1	6	4	1	3
Gastrointestinal	16	22	17	16	18	4	4	6	5	6	4	6	5	5	5	2	2	1
Respiratory (including pulm hemorrhage)	17	12	5	4	11	6	15	7	9	7	4	6	4	3	6	5	5	3
Cardiovascular	5	6	3	6	1	6	2	2	2	1	5	2	0	2	2	0	2	3
CNS	3	7	8	4	0	1	2	0	6	2	3	0	2	2	0	4	1	0
Renal	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Other / whole body conditions (e.g. septicemia)	2	0	6	3	5	2	4	2	5	4	3	2	6	3	4	1	2	3
Injection-associated	2	6	3	5	3	2	5	1	5	5	1	0	3	0	1	1	0	0
Cause of death undetermined	8	7	6	7	11	5	6	2	3	5	1	1	3	3	3	3	3	5
Total	**122**	**141**	**110**	**106**	**122**	**51**	**45**	**32**	**46**	**34**	**47**	**51**	**56**	**50**	**62**	**50**	**47**	**41**

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Hepatic lipidosis in small & toy breed puppies

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AHL Newsletter 2021;25(1):20.

Hepatic lipidosis/microvesicular degeneration is seen in puppies in the AHL caseload, and small and toy breed dogs are over-represented (**Table 1**). Primary hypoglycemia in neonatal and juvenile small and toy breed puppies is a recognized syndrome, and a connection between anorexia, fasting hypoglycemia and fatty liver syndrome has been suggested. The inciting cause can be difficult to determine, but stressful events including chilling, malnutrition, dehydration, internal parasitism, illness, injury, weaning, and re-homing are some of the recognized triggers. Neonatal and juvenile toy and small breed puppies have relatively high metabolic needs and are at increased risk of developing hypoglycemia because of their propensity for rapid depletion of blood glucose in combination with their lower muscle mass and glycogen reserves. These puppies therefore rely more heavily on fat mobilization in the face of hypoglycemia, which can lead to substantial microvesicular hepatic lipidosis (**Fig. 1**). *AHL*

Table 1. Hepatic lipidosis diagnoses in toy/small breed puppies under 6 months of age, AHL 2007-2021.

CASE	BREED	Age	Weight	Co-morbidity	Submission Diagnosis
1	Bichon Frise	12 W	0.86 kg	Pneumonia	Diffuse hepatic microvesicular lipidosis
2	Yorkshire Terrier	10 W	0.77 kg	Acute pneumonia	Diffuse hepatic lipidosis with ketonuria
3	Toy Poodle	8 W	0.72 kg	Gastric hemorrhage	Hepatic lipidosis
4	Yorkshire Terrier	3 W	0.40 kg	Enteritis-coronavirus	Hepatic lipidosis
5	Toy Poodle	8 W	0.54 kg	Enteritis – parvovirus?	Hepatic lipidosis
6	Maltese	9 W	0.59 kg	Bronchiolitis	Severe hepatic lipidosis Moderate lymphoid depletion
7	Havanese x Poodle	3.5 W	N/A	Inappetence	Microvesicular hepatic steatosis

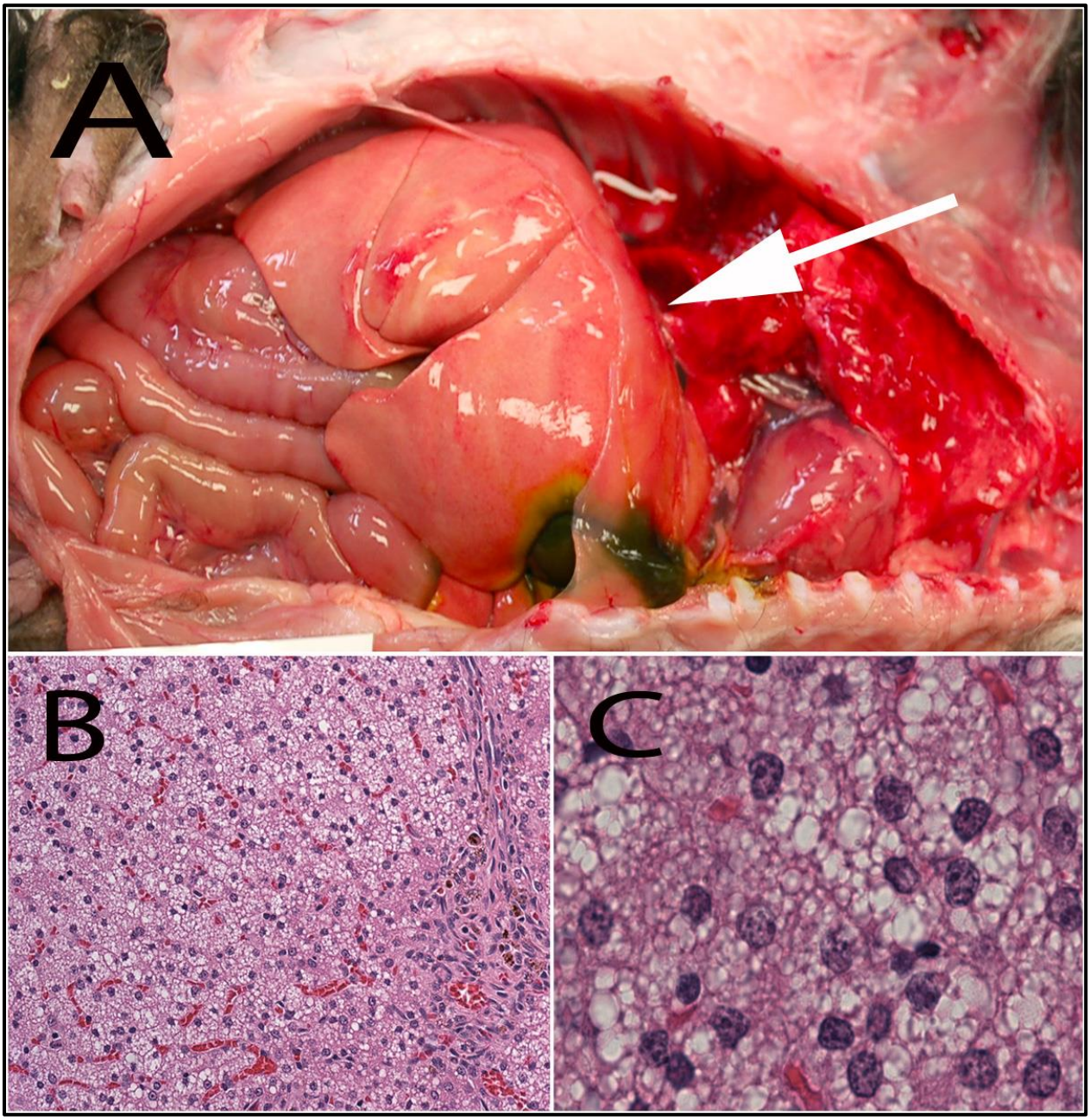

Figure 1. A. Hepatic lipidosis (arrow) in a toy breed dog. The liver is tan, greasy, and will often float in water or formalin. B and C are histologic sections showing hepatocellular microvesicular lipidosis. (H&E)

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Making sense of the toxin screen

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AHL Newsletter 2021;25(1):22.

What happens when toxicity is suspected, but there is limited or no history of exposure to a poison and the clinical presentation, laboratory data and postmortem findings do not provide any directions?

The AHL toxicology section offers several highly sensitive tests for screening of biological specimens, feed and suspect materials for the presence of toxins. These techniques include specific tests (e.g. bromethalin, ethylene glycol), as well as general methods for identification of unknown substances. The most comprehensive of these is the "toxin screen" (toxiscr). This is a qualitative multi-residue method that combines gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the identification of targeted (known) and non-targeted (unknown) substances. The readers are referred to the AHL LabNote 59 for a list of compounds that can (and a few that cannot) be identified by this screen (1). However, the GC-MS component also includes an "open screen" for unknown volatile substances. These are identified by matching an unknown compound's spectrum with a mass spectral library that contains approximately 200,000 chemicals (NIST/EPA/NIH Mass Spectral Library - NIST08); additional substances can be subsequently added. This method is an excellent first option to investigate exposure to unknown substances; however, it cannot identify all possible toxins and it has limitations that are important to understand:

- **The extraction method determines the presence or absence of the substance of interest in the sample**. There are many ways to obtain a liquid extract from the sample in order to run it through the instrument. Depending on physical-chemical structure, the solvents used in a particular method may or may not extract or retain the substance of interest. Because toxicology labs have their preferred extraction protocols, they may be able to "see" different substances in the same sample.
- **Results are qualitative** (adequate to confirm exposure); however, quantification is possible provided a standard (the purified substance) is available. This would require additional validation and would increase costs.
- Since extracts from biological samples consist of complex mixtures of molecules, **interpretation of chromatograms requires great expertise** to separate substances that are normal components of the sample (e.g. fatty acids), or background environmental contaminants from those that are potentially significant.

As a matter of example, here are a few substances that have been identified in our laboratory with the toxin screen and the interpretation provided in the context of the (often times limited) available information:

GC-MS open screen		
Identified substance(s)	Notes on case presentation	Interpretation
Caffeine and glyceril-tricaprylate (Tricaprylin)	17 dead seagulls. Submitted stomach contents (coffee beans noted) and liver.	Likely of no clinical significance. Caffeine was expected due to identification of coffee beans in the gastric content. Tricaprylin is used as a fragrance ingredient, solvent, and emollient in care products. No toxicants identified in liver.

GC-MS open screen		
Propofol	9-year-old canine. No history provided.	Pentobarbital was also found in the sample. This combination of drugs is commonly used in anesthesia.
Juvabione	Sudden death of multiple horses. Samples of bedding (common to all horses) submitted.	Probably unrelated. Juvabione belongs to a family of terpenoids present in some woods (conifers).
Etofenprox	8-year-old DSH. Post-mortem case. Interstitial pneumonia. Suspect toxicity.	Potentially toxic. This is a pyrethroid insecticide mainly excreted by glucuronidation. Since this process is limited in this species, formulations for dogs can be toxic to cats. Detailed history needed for further interpretation.

GC-MS volatile targeted compounds (included on the list of standards)		
Identified substance(s)	Notes on case presentation	Interpretation
1,1-Dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) Present at trace levels (below quantification limit).	Mature male Bald Eagle picked up alive but died before reaching the rehabilitation center. Fair to good body condition, no visible lesions. The crop was distended with recently ingested mammalian skeletal muscle.	Unlikely to be the cause of acute death (i.e. background exposure). DDE is the breakdown product of the insecticide DDT (banned in the US since 1972), which persists in the environment.
Pentobarbital (roquefortine also detected by LC-MS/MS)	Bald Eagle found on the ground unresponsive. Finder mentioned "it dropped out of sky". Obtunded. Fluid/mucus filled crop. After gastric lavage, recovered within 2 days.	These findings are significant. Suggest secondary exposure due to ingestion of tissues possibly from an improperly disposed animal euthanized with barbiturates. Roquefortine is a fungal metabolite found in moldy foods that may cause neurotoxicity at high concentrations in some species.

LC-MS/MS non-volatile targeted compounds (included on the list of standards)		
Identified substance(s)	Notes on case presentation	Interpretation
Methomyl (2)	5-month-old indoor/outdoor healthy male DSH found dead. On PM, the stomach was filled with 40 mL	Significant finding; presumably the cause of death. Methomyl is a broad-spectrum carbamate insecticide with anticholinesterase activity.

LC-MS/MS non-volatile targeted compounds (included on the list of standards)		
	of soft brown, slurry-like feed material showing blue/green discoloration.	
Imidacloprid (3)	High mortality was observed in a barn housing 4-day-old broiler chickens. Affected chicks were in ventral recumbency, appeared somnolent, with closed eyes and neurological signs consistent with leg paralysis.	Imidacloprid is particularly toxic to birds. It is a group 4 neonicotinoid insecticide to control the "darkling beetle" in poultry facilities. The pharmacological and toxic effects involve activation of nicotinic acetylcholine receptors.

AHL

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