Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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Editor: **Maria Spinato,** DVM, DVSc, Diplomate ACVP, MBA
Editorial Assistants: **Helen Oliver, Sofija Jelacic**

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. *All material is copyright 2023. Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

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Mailing address & contact information:
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
To receive an **electronic copy of this Newsletter**, please send your email address to us at holiver@uoguelph.ca

Update from the Director

The view from the Director's office

AHL is often asked to develop a new test for diseases that may be emerging, rare or challenging to diagnose. In each case, specific criteria must be considered before a new test is offered to clients. These criteria include: resources required to validate the test; availability of positive and negative samples for assay controls and the validation process; significance of the disease to the particular animal sector; and market volume. Since AHL is accredited to the ISO/IEC 17025:2017 standard by Standards Council of Canada, in addition to accreditation by the AAVLD, there are stringent requirements that need to be followed whenever a new test is added in order to ensure compliance with our rigorous quality system program.

Validation of a new test requires completion of an internal or inter-laboratory comparison using known positive and negative samples. Performance characteristics of the test (sensitivity, specificity, accuracy, reproducibility) are determined, and a decision is made regarding whether the test is "fit for use". Resources required for the validation step include staff time and supply costs, typically $3,000-$5,000 per test. If a test requires full accreditation, additional costs are incurred, including the purchase and performance of annual proficiency tests.

Therefore, the decision to develop and offer a new test depends upon a balance between resource availability, and the industry's needs and willingness to submit sufficient samples to warrant maintaining the test in AHL's inventory. For those tests that don't meet these criteria, there is always the option to refer it to another laboratory that is already performing the test. Please contact AHL Client Services if you are looking for a test that is not listed in AHL's User's Guide and Fee Schedule, and we will do our best to find a laboratory that can conduct the test for you.

And on that note, please check out the list of new tests developed in 2022 in this issue!

Maria Spinato, Director

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

Specimen Reception update

Jim Fairles

Animal Health Laboratory, University of Guelph, Guelph, ON

A Reminder to our Veterinary Clients that we must abide by the VCPR

The Animal Health Laboratory does not have a veterinary client patient relationship (VCPR) with owners, and thus cannot provide advice, results or invoices directly to your clients (owners) without your consent.
https://www.uoguelph.ca/ahl/ahl-client-communications-and-veterinarian-client-patient-relationship
We would be glad to discuss any testing related questions with you - the veterinarian. If an owner does contact us, we must refer them back to you as their veterinarian to provide testing advice. We are glad to discuss any testing related questions with you at any time.

Jim Fairles, Client Services Veterinarian.
jfairles@uoguelph.ca

Milk submissions to the AHL Milk Bacteriology Laboratory that also require testing at the Agriculture and Food Laboratory (AFL) Dairy Analysis Section

For milk submissions, please note that the AHL Mastitis lab requires a minimum of 5mL of milk for bacterial and bulk tank culture and/or bacterial counts. Should you also require testing for somatic cell counts or BactoScan (tests performed at AFL - Dairy Analysis), you will need to submit an additional 20mL of milk to complete testing for either SCC or BactoScan (40 mL if both required).

Example 1 = milk submitted to AHL for mastitis culture + SCC = minimum 25 mL milk required (equivalent to 2 yellow-capped vials)
Example 2 = milk submitted to AHL for mastitis culture + SCC + BactoScan = minimum 45 mL milk required (equivalent to 3 yellow-capped vials)

Please note: Milk requiring SCC testing should be fresh, never frozen.
Milk for mastitis culture and BactoScan cannot have any additives or preservatives
Milk should always be kept cool and sent to the lab testing within 72 h of collection.
Freezing is recommended only if samples will not be delivered to the lab within 72 h of collection.

If there are any questions, feel contact the AHL Bacteriology lab at mastitis@uoguelph.ca or 519-824-4120 ext. 54528. For questions related to tests performed at the Dairy Analysis laboratory, contact 519-824-4120 ext. 57273 or email Looknauth Ramshaoi at lramsaho@uoguelph.ca .

AHL New Tests Developed in 2022

Helen Oliver

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

AHL Newsletter 2023;27(1):4.

TEST METHOD	**CODE**	**SPECIES**
IAPD (Ontario Interactive Animal Pathogen Dashboards) membership (Tableau viewer license) charge.	iapd	Avian, Bovine, Canine, Caprine, Equine, Feline, Other, Ovine, Porcine, Chicken, Turkey
Lactococcus garvieae - qPCR	lgrvpcr	Other
Mycoplasma bovis semen - PCR	nbsemen	Bovine
Parelaphostrongylus tenuis (meningeal worm) PCR and sequencing identification	parepcr	Bovine, Caprine, Equine, Other, Ovine
Rabbit Hemorrhagic Disease Virus 2 – PCR	rhdvpcr	Other
Salmonella Dublin antibody ELISA (now also in fresh milk and bulk-tank milk) – see article on page 14 "*Salmonella* Dublin testing in milk now available"	salmdel	Bovine

OAHN Update – March 2023

Mike Deane, Tanya Rossi

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

This winter, OAHN was happy to host its annual meeting, bringing together all of our networks to share their successes from the past year, and discuss plans for the year ahead. We had an informative and interesting plenary session on vector-borne disease surveillance in a changing world, presented by Dr. Katie Clow and Ms. Valentina Rodriguez. It was an illuminating experience to see what the OAHN networks have accomplished during the past year, and we are looking forward to an even more productive 2023. To view any of our network reports and research projects, go to OAHN.ca and navigate to the species of interest.

New Resources

- OAHN Poultry Project: Developing a response plan for effective infectious laryngotracheitis outbreak management in Ontario
- Rabbit Hemorrhagic Disease Surveillance Project
- OAHN Wildlife Project: Identifying changes in *Leptospira interrogans* prevalence and serovars in wildlife in Ontario
- Infographic: Information sources for smallholder swine producers and pet pig owners
- Aquatic Network Research Project: Biosecurity for aquatic animal facilities – content development for a website and workshop, thinkific platform and booklet

Video: Need to Know: Rabies in Pets

The OAHN Companion Animal Network, in conjunction with Tivoli Films, created an interesting, fact-filled whiteboard video illustrating rabies and the process of importing dogs from other countries. A must-watch for anyone wondering how rabies works, and why there are import requirements for dogs entering Canada.

Check out the video and many rabies resources here: https://www.oahn.ca/resources/video-need-to-know-rabies-in-pets/

New Reports

Most OAHN networks create reports once per quarter. To view any of the veterinary reports below, please click on the OAHN icon for each network, or go to OAHN.ca and navigate to the species in which you are interested.

- Turkey Viral Hepatitis (TVH) vs. Turkey Hepatitis Reovirus (THRV)
- Aortic rupture: A cause of sudden death in turkeys
- Poultry Veterinary Survey Highlights – Q4 2022
- Research

- Disease surveillance discussion
- Animal Health Laboratory syndromic surveillance
- Animal Health Laboratory diagnostic reports
- Ontario slaughter statistics
- CanSpotASF surveillance update

- OAHN survey: tick concerns, toxicoses
- *Echinococcus multilocularis* refresher
- RHDV2 subsidized testing available
- Rabies update
- NEW! Rabies whiteboard video
- Got blasto cases?
- More pet pig resources
- OAHN survey: CIRDC, parvovirus

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Selected zoonotic pathogens and diseases in Ontario identified at the AHL in 2022

Tanya Rossi

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(1):7.

The term One Health, "an integrated, unifying approach to balance and optimize the health of people, animals and the environment"[1], is a relatively new one in medicine; however, the contribution of veterinarians to public health dates back hundreds of years. This contribution has taken many forms, including using comparative physiology and anatomy to further human health, informing policies involving food safety and ecosystem health, and detection of zoonotic pathogens, among others. AHL participates in many of these initiatives, but our primary contribution is the surveillance and annual reporting of zoonotic pathogens identified at our laboratory (**Tables 1 & 2**).

Case numbers for most zoonotic pathogens isolated or identified by the AHL in 2022 are relatively unchanged from the previous year. *Blastomyces dermatitidis* cases have increased in canines from 5 in 2021 to 13 in 2022. Avian West Nile virus cases have also increased this year, rising from 11 in 2021 to 26. Isolation of *Methicillin-resistant S. pseudintermedius* (MRSP) in canines decreased in 2022, as well as the number of positive serology submission for *Borrelia burgdorferi* in canines and equines.

The percentage of animals identified as positive for leptospirosis was roughly unchanged in cattle and dogs in 2022, and the total number of submissions tested was about the same as 2021. The percentage of positive leptospirosis cases increased in equines and swine, and the number of submissions was relatively unchanged in swine and slightly increased in equines. 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 except horses may be routinely vaccinated for leptospirosis. Test results submitted under monitoring programs are not included. *Brucella canis* results shown are positive on the 2ME-RSAT (**Table 1**). *AHL*

Table 1. Number of cases for selected zoonotic pathogens isolated and/or identified at the AHL, 2022.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2022	2021
Ascarids (incl *T. canis, T. cati, T. leonina, Baylisascaris* sp.)		4	4			63	1	21	4	13	**110**	**100**
Blastomyces dermatitidis								13			**13**	**5**
Bordetella bronchiseptica	6	30	7					1	1	6	**51**	**63**
Borrelia burgdorferi (Lyme disease), serology			2					14			**16**	**36**
Brucella sp. (non-*abortus)*								90			**90**	**131**

Campylobacter coli/ jejuni/ fetus subsp. *fetus*	1	1		11				2			**15**	**9**
Chlamydia sp.				9	15						**24**	**22**
Clostridium difficile		1	1								**2**	**2**
Coxiella burnetii (Q fever)	8			26	22						**56**	**56**
Cryptococcus sp.								2	1		**3**	**0**
Cryptosporidium sp.	169			3	6			2		13	**193**	**201**
Eastern equine encephalitis virus	1									2	**3**	**8**
Echinococcus multilocularis								5		2	**7**	**2**
Giardia sp.	8				1			31			**40**	**31**
Listeria monocytogenes	8			5	11					1	**25**	**21**
Methicillin-resistant *Staph aureus* (MRSA)		2	1			1		2		1	**7**	**5**
Methicillin-resistant *S. pseudintermedius* (MRSP)								47	2	6	**55**	**90**
Rabies virus											**0**	**4**
Salmonella enterica	66	93	5	8		46	8	1		13	240	**245**
Streptococcus suis	2	195				5	2				**204**	**186**
Streptococcus equisimilis	3	43	22		1			2		1	**72**	**64**
Streptococcus zooepidemicus		1	152	1	1			1		1	**157**	**168**
Toxoplasma sp.				15	3						**18**	**18**
Verotoxigenic *E.coli* (VTEC)	1				2						**3**	**6**
West Nile virus			1							26	**27**	**14**
Yersinia enterocolitica		2									**2**	**4**
Total											1433	1491

Table 2. *Leptospira* spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL, 2022.

Leptospira spp. serovar	**Bovine**	**Swine**	**Equine**	**Canine**	**Other**
L. autumnalis	22	5	23	59	
L. bratislava	20	5	29	39	
L. canicola	16	4	13	45	
L. grippotyphosa	4	2	6	28	
L. hardjo	23	4	11	15	
L. icterohaemorrhagiae	21	5	22	47	
L. pomona	20	5	17	38	
IHC or PCR-positive	0	2	1	7	
Positive/tested cases	**45/148**	**7/33**	**32/48**	**87/171**	**0/5**
% pos	30.4%	21.2%	66.7%	50.9%	0%
% pos, 2022/2021	30/31%	21/14%	67/54%	51/58%	0/50%

Reference

1. Adisasmito WB, Almuhairi S, Behravesh CB, Bilivogui P, Bukachi SA, et al. (2022) One Health: A new definition for a sustainable and healthy future - One Health High-Level Expert Panel (OHHLEP). PLoS Pathog 2022;18(6): e1010537. https://doi.org/10.1371/journal.ppat.1010537

Detection of ionophores in animal tissues

Felipe Reggeti, Nick Schrier, David MacKay

Animal Health Laboratory, University of Guelph, Guelph, ON (Reggeti, Schrier), Agriculture and Food Laboratory, University of Guelph, Guelph, ON (MacKay)

AHL Newsletter 2023;27(1):10.

Ionophores are antibiotics approved as feed additives for certain species due to effective anticoccidial properties, as well as enhancement of feed efficiency and rate of weight gain. These drugs are safe when used according to label specifications; however, when the dose exceeds the recommendations (e.g., feed mill mixing errors) or the medicated feed is inadvertently consumed by off-target species, high morbidity and mortality may occur.

The diagnosis of ionophore toxicosis is based upon history of exposure, identification of compatible clinical signs and histopathological lesions, and quantitative analysis of feed (dose) and gastrointestinal (GI) contents (exposure). On occasions, the level of exposure is unknown, the available feed samples are not representative, and GI contents are not collected during postmortem examinations; therefore, testing fresh tissues provides the only opportunity for further assessment. The main limitations with this approach are that ionophores are rapidly excreted from the body, and detailed information on pharmacokinetics for interpretation of the significance of tissue levels is limited. As a consequence, **estimation of the ingested dose by extrapolation from tissue levels is difficult, if at all possible, and unfortunately, not practical**. **Thus, the main objective of testing tissues for ionophores should be to confirm exposure**.

Detection of ionophores in tissues is particularly helpful when investigating incidents involving non-target/susceptible species (e.g., horses), because any residues in biological specimens would be considered significant. In a series of cases looking at the diagnostic value of monensin concentration in tissue from horses following toxicosis, exposure was confirmed by toxicological analysis of serum, urine, blood, liver, heart and stomach content; however, there was no correlation between tissue concentrations and clinical outcome.

The Agriculture and Food Laboratory (AFL) at the University of Guelph offers analytical testing of selected antimicrobials in animal tissues for regulatory monitoring. **The "Veterinary Drug Screen" (LC-MS/MS) is a multi-target analysis (MTA) that includes the ionophores monensin, narasin, lasalocid and salinomycin**, as well as other antibiotics and non-ionophore anticoccidial drugs (**Table 1**). The high sensitivity of the test makes it suitable for food safety analysis, but it can also be used in cases of suspected toxicosis. Samples for toxicological investigations may be submitted through the Animal Health Laboratory (AHL). The method is validated for fresh/frozen liver (poultry) and kidney (ruminants and swine), as well as skeletal muscle (all species). For questions, please contact the AHL Toxicology laboratory: ahltoxi@uoguelph.ca. *AHL*

Table 1. Veterinary Drug Screen listing compounds detected with minimum quantification limits (MQL)

Compounds	MQL (ng/g, ppb)	Compounds	MQL (ng/g, ppb)	Compound	MQL (ng/ g, ppb)
Abamectin B1a	25	Erythromycin	10	Sulfabenzamide	25
Albendazole-2-aminosulfone	125	Fenbendazole	25	Sulfacetamide	25
Amoxicillin	6	Florfenicol	50	Sulfachloropyridazine	25
Ampicillin	3	Flunixin	5	Sulfadiazine	25
Amprolium	50	Gamithromycin	25	Sulfadimethoxine	25
Buqinolate	120	Halofuginone	5	Sulfadoxine	25
Cephapirin	25	Ivermectin B1a	25	Sulfaethoxypyridazine	25
Chloramphenicol	1.3	Josamycin	10	Sulfaguanidine	25
Chlortetracycline	25	Ketoprofen	125	Sulfamerazine	25
Ciprofloxacin	5	Lasalocid	25	Sulfamethazine	25
Clenbuterol	1.3	Levamisole	25	Sulfanilamide	50
Clindamycin	25	Lincomycin	6	Sulfapyridine	25
Clopidol	50	Meloxicam	25	Sulfaquinoxaline	25
Cloxacillin	3	Meduramicin	50	Sulfathiazole	25
Closantel	100	Monensin	13	Tetracycline	30
Danofloxacin	25	Moxidectin	25	Thiabendazole	25
DCCD	50	Nicarbazin	15	Thiabendazole (5-OH)	25
Decoquinate	25	Narasin	30	Thiamphenicol	25
Derquantel	5	Novobiocin	50	Tildipirosin	50
Desethylene ciprofloxacin	6	Oxytetracycline	30	Tilmicosin	25
Dexamethasone	6	Penicillin G	13	Toltrazuril Sulfone	63
Diclazuril	50	Phenylbutazone	6	Tulathromycin A	50
Dinitolmide	80	Pirlimycin	25	Tylosin	50
Doramectin	25	Ractopamine	10	Tylvalosin	6
Doxycycline	6	Robenidine	6	Virginiamycin M1	6
Enrofloxacin	5	Salinomycin	50	Zeranol	6
Eprinomectin B1a	25	Sarafloxacin	15	Zilpaterol	1.3

References

1. Hall JO. Ionophores. In: Plumlee's Clinical Veterinary Toxicology. Plumlee, KH, ed. Mosby, 2004:120-127.
2. Puschner B, et al. Serum, milk, and tissue monensin concentrations in cattle with adequate and potentially toxic dietary levels of monensin: pharmacokinetics and diagnostic interpretation. J Vet Pharmacol Ther 2016;39(4):363-372.
3. Bautista AC, et al. Diagnostic value of tissue monensin concentrations in horses following toxicosis. J Vet Diagn Invest 2014;26(3):423-427.

Babesia odocoilei infection associated with acute mortality in farmed elk in Ontario

Rebecca Egan, Felipe Reggeti, Amanda Mansz, Hugh Cai

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(1):12.

In October of 2022, a diagnostic investigation was performed in a herd of approximately 180 farmed elk in Ontario to explore the cause of acute death and illness in a subgroup of animals that had been brought in from Western Canada in late summer. One mature female had been found dead, and clinical examination of a bull revealed lethargy, hindlimb weakness and the voiding of red urine. A field postmortem was performed on the female, and an EDTA blood sample and formalin-fixed tissues were submitted to the AHL.

The CBC showed marked anemia (Hct: 0.11 L/L; RI:0.30 - 0.41 L/L) that was macrocytic (MCV: 50 fL; RI: 42.1–44.6 fL), along with neutropenia (0.45 x 109/L; RI: 0.86 - 5.29 x 109/L). Examination of blood smears revealed small rounded to pear-shaped intra-erythrocytic inclusions, observed both individually and in pairs, compatible with *Babesia* spp. (**Fig. 1**). DNA was extracted from the positive blood sample, and sequencing of PCR products from the 18S rRNA showed 100% similarity (435/435 bp) with *Babesia odocoilei*. Serum *Anaplasma* antibody ELSIA was also performed, and the result was negative. Other signs of regeneration were not obvious on the blood smear. Although biochemistry profile and urinalysis were not performed, the findings of marked anemia, intra-erythrocytic inclusions, and red urine were highly suggestive of intravascular hemolysis and hemoglobinuria.

Microscopic examination of tissues from the female identified accumulation of granular red-pink pigment in many renal tubules, compatible with either hemoglobin or myoglobin (**Fig. 2**), and periacinar to occasionally mid-zonal hepatocellular necrosis typical of hypoxic injury (**Fig. 3**). Frequent single cell death was present in mid-zonal and periportal regions (**Fig. 3**), prompting consideration of copper toxicosis. Therefore, hepatic and renal copper quantification was performed; copper levels were not elevated. Tissues from a second field postmortem case were submitted, revealing similar renal and hepatic lesions. However, single cell death in liver was not present. Based on the clinical presentation, the possibility of bacillary hemoglobinuria caused by *Clostridium haemolyticum* was a consideration; however, hepatic lesions typical of this disease were not identified.

The bull subsequently died and was submitted to the AHL for postmortem. On examination, the bull had pale mucous membranes and was emaciated, with absence of internal fat stores and serous atrophy of fat in bone marrow. The liver was small, and renal cortices had a slightly darkened brown-black appearance. Examination of smears prepared from drops of blood collected from small vessels in the ears and tail revealed intracellular organisms similar to those observed in the initial blood sample. Microscopically, the liver had extensive regions of periacinar hepatic necrosis, with remaining hepatocytes exhibiting atrophy and cytoplasmic vacuolation in periportal regions. Pigmentary nephrosis and tubular degeneration were present in kidney, consistent with intravascular hemolysis.

Considering the combination of findings in this diagnostic investigation, together with similar reports in the literature, it was determined that these animals succumbed to an acute hemolytic crisis, presumably

incited by latent *Babesia* infection compounded by the stress of relocation, negative energy balance, and rutting season. Babesiosis is a tick-borne disease, and the primary vector is the blacklegged tick (Ixodes scapularis) which is present in Ontario, and also serves as the vector for *Borrelia burgdorferi*, the cause of Lyme disease. In this case, it is not clear how these elk acquired the infection, and given that all four of the affected animals were from the group that were recently brought in from Western Canada, it is possible that these animals were infected prior to arrival in Ontario. *AHL*

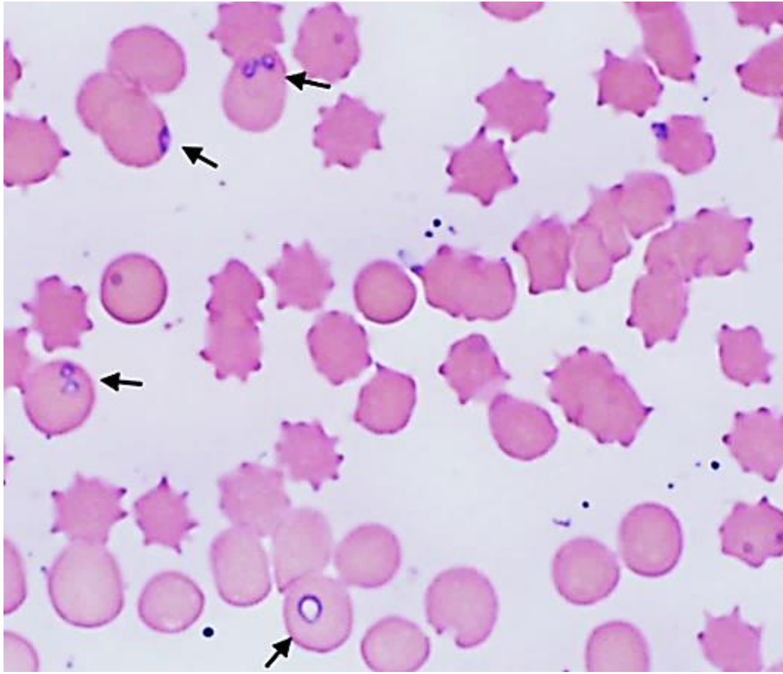

Figure 1. Blood smear demonstrating small rounded to pear-shaped intra-erythrocytic inclusions, observed individually and in pairs (arrows), compatible with *Babesia* spp.

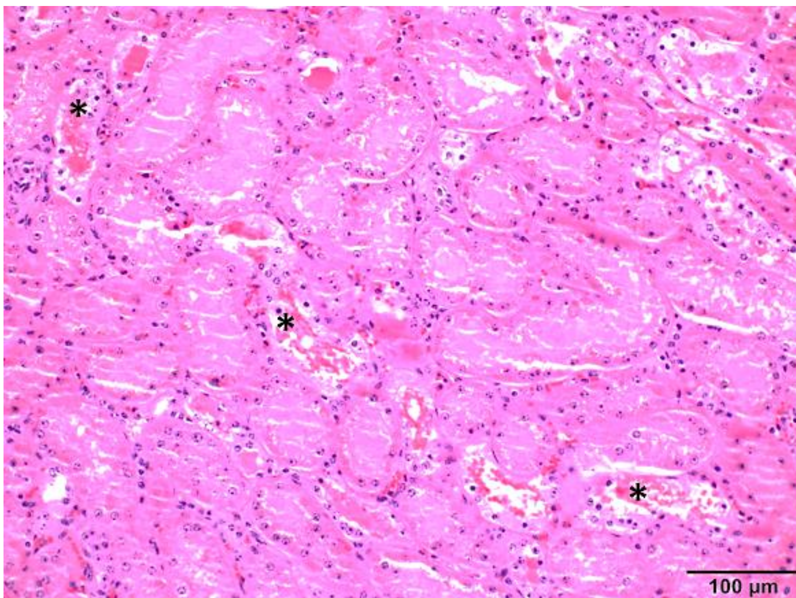

Figure 2. Microscopic section of kidney (H&E, 20x) demonstrating luminal accumulation of granular red-pink pigment in renal tubules (*).

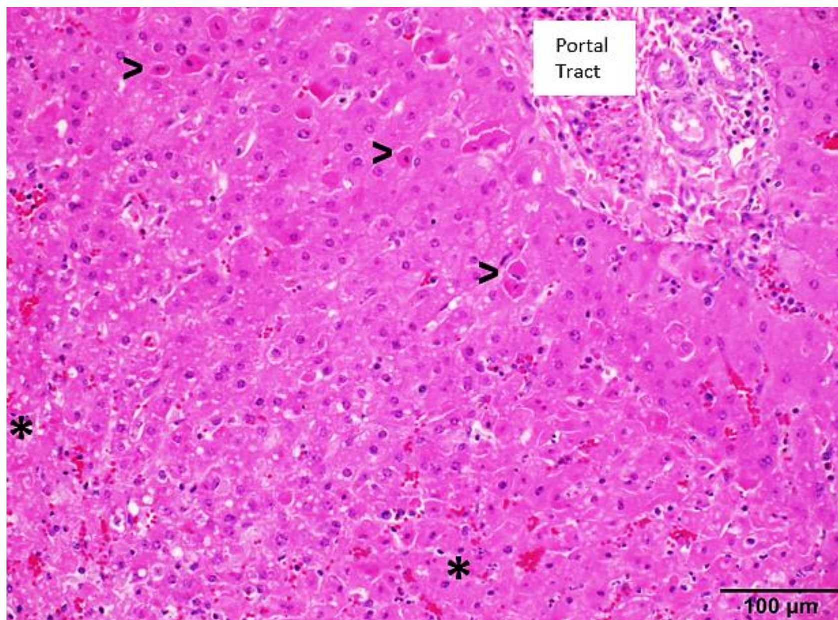

Figure 3. Microscopic section of liver (H&E, 20x) demonstrating periacinar to mid-zonal hepatocellular degeneration (*) and single cell death among periportal hepatocytes (>).

References
1. Pattullo KM, et al. *Babesia odocoilei* infection in a Saskatchewan elk (*Cervus elaphus canadensis*) herd. J Vet Diagn Invest 2013 Jul;25(4):535-40.
2. Mathieu A, et al. *Babesia odocoilei* as a cause of mortality in captive cervids in Canada. Can Vet J. 2018 Jan;59(1):52-58.

Salmonella Dublin testing in milk now available

Jim Fairles, Davor Ojkic

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(1):14.

The AHL has verified the suitability of PrioCheck *Salmonella* Dublin antibody ELISA for milk samples. This verification was performed using: (i) milk and serum samples received from Ontario practitioners; and (ii) an interlaboratory comparison with another AAVLD-accredited laboratory (Animal Health Diagnostic Centre, Cornell University). The results of this comparison confirmed that this antibody ELISA is a robust and reliable test for milk as well as serum. Therefore, the AHL is now accepting both milk (individual animal milk samples and string or bulk tank milk samples) and serum (individual serum samples) for *Salmonella* Dublin antibody ELISA testing.

Please note that this test is very sensitive; consequently, milk carry-over from one cow to the next can result in a false positive test result. Therefore, careful attention to milking order and verification of positive milk results using serum testing are important when testing individual animal milk samples.
AHL

Porcine sapovirus: An emerging pathogen contributing to swine diarrhea

Josepha DeLay, Davor Ojkic

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(1):15.

Porcine sapovirus (PSaV) has been associated with diarrhea in pigs of all ages, commonly in mixed infection with other viral, bacterial, and / or protozoal pathogens. The virus has been detected in swine worldwide, and in both symptomatic and asymptomatic pigs, although a recent study has demonstrated higher viral loads in animals with clinical diarrhea. PSaV was first detected in 1980 in the United States in a diarrheic piglet co-infected with rotavirus and another undetermined virus. Subsequently, enteritis was experimentally reproduced in gnotobiotic piglets orally infected with the virus.

PSaV is a member of the Calicivirus family. Currently, 8 genogroups of the virus are known, and genogroup III is predominant. Recombination within and between genogroups, and genetic drift are common viral adaptation strategies among sapoviruses. It should be noted that PSaV is distinct from the similarly named porcine sapelovirus (PSV) which causes neurologic disease in pigs.

Recent research has indicated that PSaV may be an emerging pathogen, and in some herds, can be a significant contributor to enteric disease in young pigs. Using whole genome sequencing, PSaV has been identified in feces from pigs of various ages with clinical diarrhea, often in mixed infection with other enteric pathogens, but occasionally as the sole pathogen identified.

PSaV has recently been detected in nursing piglets with diarrhea from an Ontario herd. The piglets were negative for other common enteric pathogens (rotavirus, PEDV, PDCoV, TGEV, bacteria, coccidia) based on PCR, culture, and histopathology results. Histologic features of sapovirus enteritis are similar to those of other enteric viruses (rotavirus, porcine coronaviruses), characterized by villus atrophy and epithelial attenuation at villus tips. In experimental infections, the anatomic distribution of lesions differed from other enteric viral pathogens in that lesions were most prominent in duodenum and less severe in jejunum and ileum. Similar distribution has not been described in recent literature reports of natural infections.

Knowledge of the relative importance of PSaV in swine enteric disease is still incomplete. A potential role for the virus should be considered in swine diarrhea cases, especially in nursing and weaned pigs, and when other enteric pathogens are not detected. Currently, testing for PSaV by PCR and *in situ* hybridization (ISH) is carried out at a laboratory external to the AHL. However PSaV PCR is being developed and will be available at the AHL in the near future. PSaV can be detected in both symptomatic and asymptomatic swine, and mixed infections with other pathogens are common. With these features in mind, it is important to interpret PSaV test results in the context of test results targeting other common enteric pathogens. Formalin-fixed samples for histopathology should include duodenum, as well as jejunum, ileum, and colon, due to the predominance of duodenal lesions noted in experimental PSaV infections. *AHL*

Reference

1. Nagai M et al. Porcine sapoviruses: Pathogenesis, epidemiology, genetic diversity, and diagnosis. Virus Res 2020; https://doi.org/10.1016/j.virusres.2020.198025

Piglet meningoencephalitis attributed to PCV-3 infection

Amanda Mansz

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2023;27(1):16

As previously published in AHL Newsletter 2021;25(2):14 (https://www.uoguelph.ca/ahl/porcine-circovirus-3-pcv3-results-ahl-2018-2021), retrospective studies have confirmed that porcine circovirus-3 (PCV3) has been present but undetected in swine herds for many years. Association of this virus with clinical disease is a recent event. Although PCV3 belongs to the same virus family as PCV2, vaccines do not provide cross-protection between the 2 viruses, and tests for each virus are distinct and will not cross-react. PCV3 has been associated with an increase in mummified and stillborn fetuses, weak neonates, as well as poor growth in older pigs. Previously documented cases highlighted histologic lesions in PCV3-infected fetuses and pigs that included lymphocytic myocarditis and myocardial fibrosis, and lymphocytic perivasculitis in various organs, especially in heart and kidney. Using *in situ* hybridization (ISH) methods, PCV3 nucleic acid can be detected in tissues, co-localized with histologic lesions.

A postmortem submission to the AHL of two 2 to 3-day-old piglets from a herd with a recent history of young piglets with unusual body conformation (pinned-back ears and humped backs) followed by signs of pneumonia and death was recently completed. A diagnosis of PCV3 infection was made by a combination of test results including: suggestive histological lesions, strongly-positive PCR detection of PCV3 in lung tissue, and ISH testing. Histological lesions in both piglets included multi-organ mononuclear perivascular cuffing, and vasculitis within sections of lung, heart, kidney, liver, stomach, intestinal mesentery, brain and spinal cord (**Fig. 1**). PCR testing of a pooled sample of lung from both pigs was positive for PCV3 with a Ct value of 19.99. A single section of brain with non-suppurative vascular lesions was sent to the Veterinary Diagnostic Laboratory of Iowa State University for ISH testing, and profound intracytoplasmic staining of both neurons and adjacent glia for PCV3 was detected. Although non-suppurative encephalitis in neonates has been documented previously, this was one of the first cases diagnosed at the AHL.

PCV3 should be considered as a rule-out for herds experiencing reproductive losses due to increased stillborn and mummified fetuses and/or weak neonates, neonates with a complaint of failure to thrive, and nursery or grow-finish pigs with poor growth and ill-thrift. Along with microscopic evaluation of a full range of tissues, useful samples for PCV3 PCR testing include pooled tissues (lung, heart, kidney) from fetuses or older pigs, in addition to fetal thoracic fluid and processing or castration fluids. *AHL*

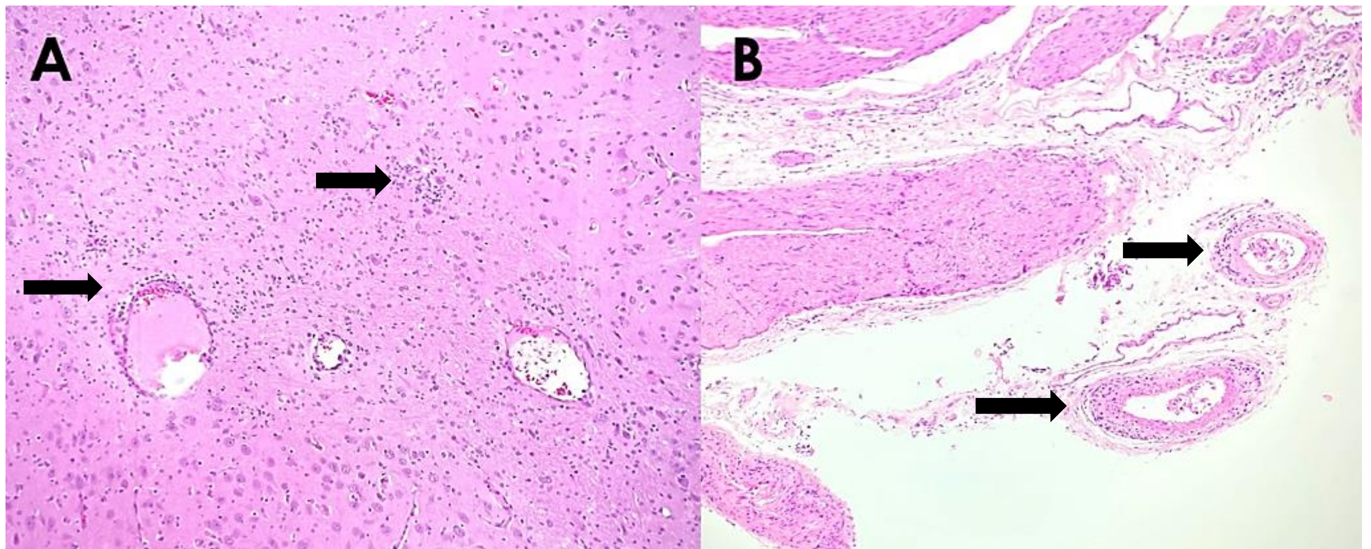

Figure 1. Histological lesions of PCV3 infection in piglets (H&E stain). **A.** Lymphoplasmacytic encephalitis of the midbrain (arrows). **B.** Arteries within the leptomeninges of the spinal cord infiltrated or cuffed by plasma cells and lymphocytes (arrows).

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Avian chlamydiosis outbreak in a breeding flock of African grey parrots

Hein Synman, Pauline Delnatte, Christopher Dutton

Animal Health Laboratory, University of Guelph, Guelph, ON (Snyman); Ontario Veterinary College (Delnatte, Dutton)

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Over a 6-week period in the early fall of 2022, a breeding flock of approximately 30 adult African grey parrots (*Psittacus erithacus*) started to experience sudden and unexplained mortalities. The first death occurred in September, and was followed by two more in early and then late October. One bird was found dead with no premonitory signs, and two birds exhibited a short preceding course of non-specific clinical signs consisting of weight loss, ruffled feathers, and lethargy. At the time of the third mortality, two additional birds had also started showing the same non-specific clinical signs. One of these birds died 3 days later. All birds were young adults (~ 8-12 years old), with no significant preceding medical history. During the summer, birds were housed in a free-flight barn with fine mesh windows, and in the winter, birds were pair-housed in cages in a heated barn. The move from summer to winter housing occurred about a week before the third mortality. The flock was considered to be a closed flock with no new birds introduced into the flock for more than 6 years. There had been no mortalities in the flock over the previous 10 years.

At the time of the third mortality, an onsite veterinary visit was scheduled, and a postmortem was performed. On external postmortem examination, this male bird was found to be in poor body condition with severe dehydration, unilateral left lenticular opacification, and a small amount of dry discharge was present around the right nostril. Internally, the liver was markedly enlarged and friable with diffuse yellow-tan mottling and fibrinous adhesions. The pericardial sac was thickened, opaque and filled with a large amount of yellow-green gelatinous exudate (**Fig. 1A**). The spleen was also markedly enlarged (~ 2 cm diameter) and friable with dark red congestion and similar pale mottling (**Fig. 1B**). The lungs were diffusely congested and edematous (**Fig. 1B**). Representative formalin-fixed and fresh tissue samples were taken and submitted to the Animal Health Laboratory for further analysis.

Bacterial culture of the spleen did not yield any significant pathogens and salmonellosis was ruled out by enriched media culture. Histological sections of the liver and spleen contained widespread coalescing foci of acute parenchymal necrosis with moderately dense mixed infiltrates of macrophages, fewer heterophils, fibrinous exudate, and karyorrhectic nuclear debris (**Figs. 2A, 2B**). Many macrophages in addition to viable marginal hepatocytes were distended by densely-aggregated fine granular pale basophilic cytoplasmic bacterial colonies. Similar fibrino-inflammatory exudate and intra-histiocytic bacteria were also present on the surface of the pericardial sac (**Figs. 2C, 2D**). These features are highly suggestive of avian chlamydiosis, a diagnosis that was subsequently confirmed by PCR testing of the spleen (Ct = 15.82). Postmortem findings in the fourth mortality were very similar. Histology was not performed in this parrot. *Chlamydia psittaci* was confirmed by PCR testing of the spleen (Ct = 16.25).

Avian chlamydiosis is a zoonotic disease that is caused by the obligate intra-cellular bacterium *Chlamydia psittaci*. Aerosol inhalation and ingestion represent the primary routes of transmission / infection, and carriers are capable of excreting the organism intermittently for several months post infection. Given that immunity post infection is short lived, intermittent shedding also often results in

rapid reinfection which can lead to prolonged recirculation of the disease in an affected population. *C. psittaci* elementary bodies can remain infectious for several months.

Clinical signs vary widely within and among individuals in an affected population, and may include: sinusitis, dyspnea, conjunctivitis, polyuria, diarrhea, lethargy, anorexia, yellow to dark green droppings, poor feathering, chronic weight loss, various neurological abnormalities (flaccid paralysis, opisthotonos, tremors, convulsions), or peracute death.

Although this group of birds was predominately housed indoors, infection was still likely related to incidental contact with wild birds, either directly through fine mesh windows or indirectly through contaminated dust, dried saliva, feathers, mucous and/or feces. The outbreak was reported to the Ontario Ministry of Health and Long-Term Care, and appropriate follow-up precautions were undertaken to limit any human contact with the remaining birds. The remaining symptomatic bird received intensive care for 5 days (supportive care and doxycycline orally) and improved quickly. The entire flock was immediately started on a 6-week course of doxycycline administered in drinking water and no additional mortality or morbidity occurred. *AHL*

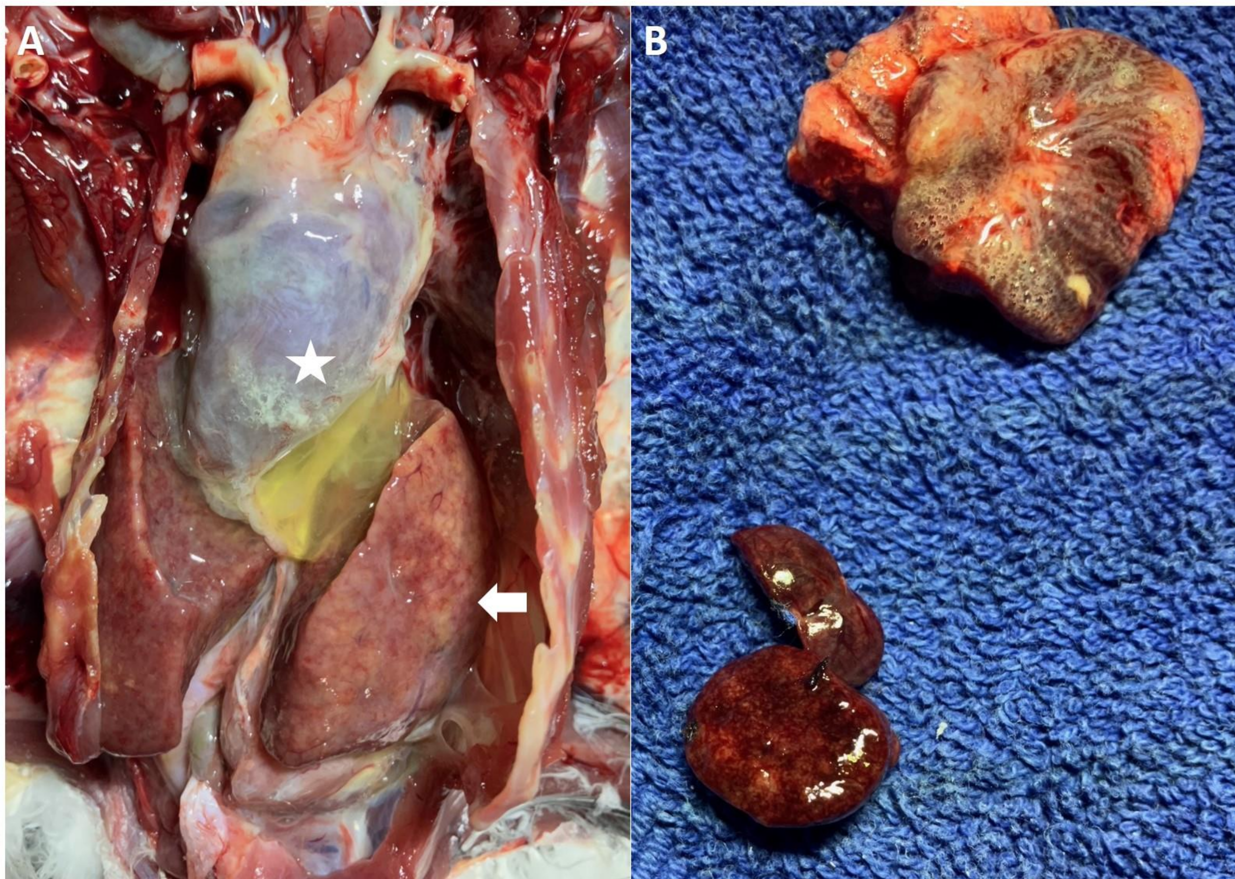

Figure 1. Postmortem findings in a 10-year-old male African grey parrot with avian chlamydiosis. **A.** In-situ coelomic organs. Hepatomegaly with rounded lobar margins and pale yellow-tan mottling (arrow). The pericardial sac is thickened, opaque and is filled with light yellow-green gelatinous exudate (star). **B.** Lung (top) and spleen (bottom). Lungs are congested and edematous; spleen is markedly enlarged with mottled and friable parenchyma.

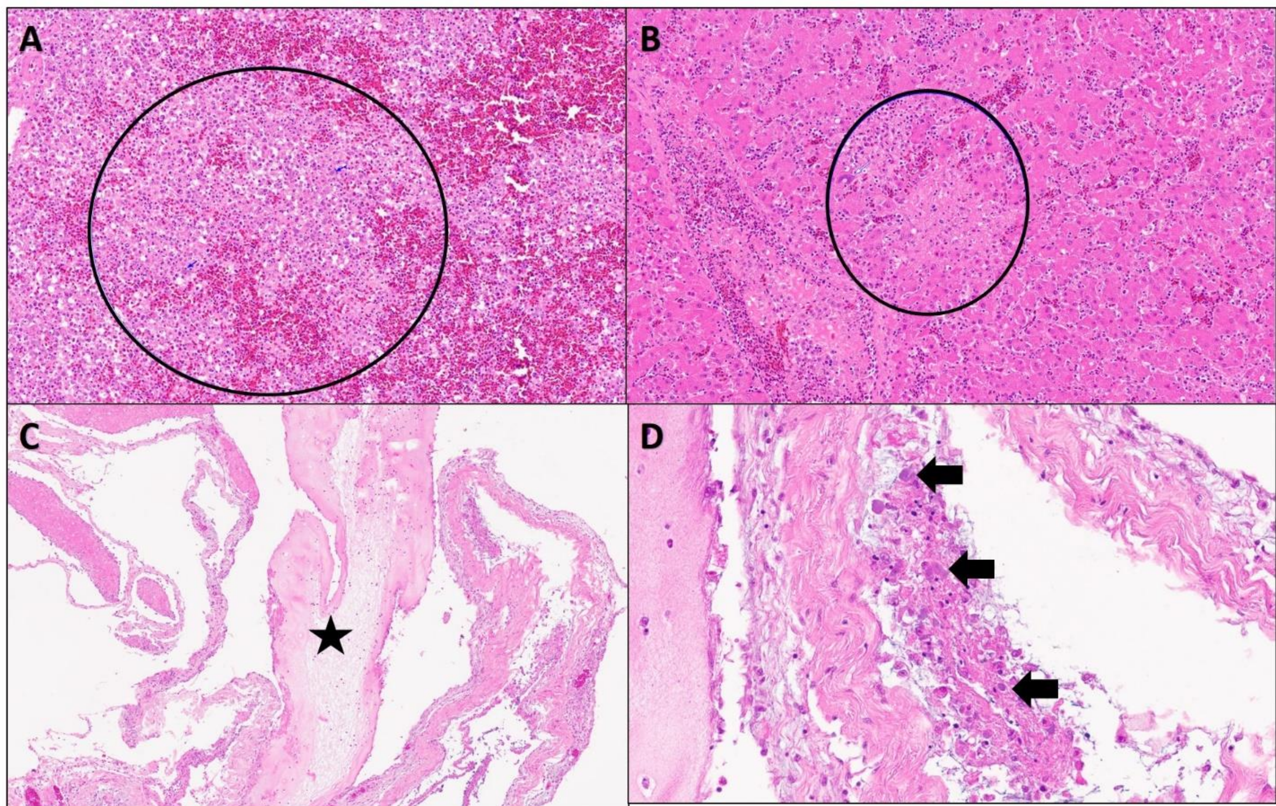

Figure 2. Histological findings in a 10-year-old male African grey parrot with avian chlamydiosis (H&E stain). **A.** Spleen contains large foci of acute parenchymal necrosis, associated with a mixed cellular infiltrate of macrophages and fewer heterophils (circle). **B.** Liver contains similar foci of necrosis and histiocytic inflammatory infiltrate (circle). **C. & D.** The pericardial cavity is filled with fibrino-cellular exudate (star) and numerous macrophages that are distended by densely-aggregated, fine granular pale basophilic cytoplasmic bacterial colonies (arrows).

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Riboflavin deficiency in broiler chickens

Andrew Brooks, Emily Martin

Animal Health Laboratory, University of Guelph, Guelph, ON

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Lameness and musculoskeletal disorders are common problems in poultry. In 2022, the AHL diagnosed multiple cases of confirmed or suspected riboflavin deficiency resulting in lameness and limb paralysis in broiler chickens.

In this series of cases, the affected birds ranged from 10 to 20 days of age, and common clinical signs included lameness, paralysis, inward curling of the toes and increased culling. Of the cases submitted for postmortem examination, the gross lesions were generally nonspecific, but common findings included curled toes (**Fig. 1)**, mild limb rotation, and evidence of wing walking characterized by lesions on the carpi and wing tips.

Histologically, the diagnostic lesions were present in large peripheral nerves such as the sciatic nerve. In most nerves, there were mild to moderate infiltrates of mononuclear inflammatory cells including lymphocytes and fewer plasma cells (**Fig. 2A**). Schwann cells exhibited hypertrophy and hyperplasia with enlargement of the nuclei, and mitotic figures were evident in many affected nerves. Axon and myelin sheath degeneration was a consistent finding (**Fig. 2B**).

Riboflavin, a water-soluble B vitamin (Vitamin B_2), is an essential cofactor for many enzymes involved in energy metabolism and cellular growth. Riboflavin supplementation is required in poultry rations, and deficiency results in chicks that are weak and slow-growing. Affected chicks are often reluctant to stand or walk, may rest on the hocks, and may walk with the assistance of the wings. A characteristic presentation is “curled-toe paralysis” where the toes of the lame birds curl inwards. Other clinical signs may include reduced growth, diarrhea and increased mortality. When detected early, riboflavin deficiency can be treated effectively with vitamin supplementation. *AHL*

Figure 1. Chick affected with “curled-toe paralysis” due to riboflavin Deficiency (photo courtesy of Dr. Anastasia Navy).

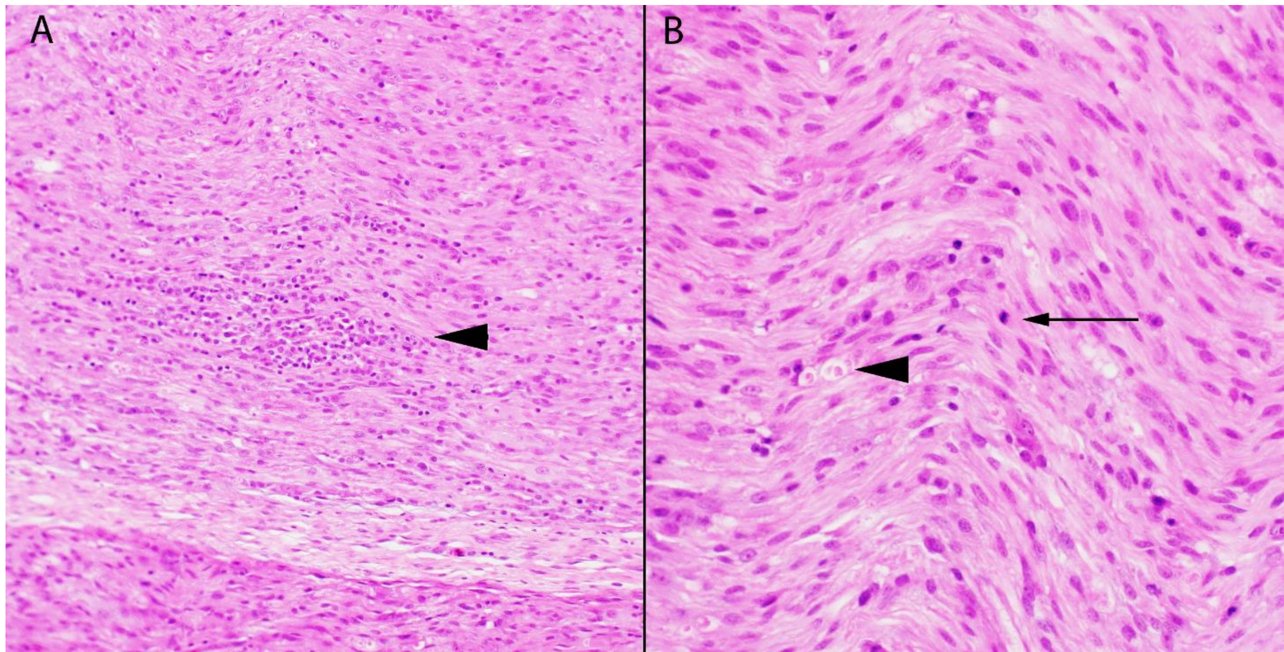

Figure 2. Lesions in the peripheral nerves associated with riboflavin deficiency (H&E stain). **A**. Mononuclear cell infiltrates (arrowhead). **B**. Schwann cell hyperplasia and hypertrophy, with mitotic figure (arrow), and axon degeneration (arrowhead).

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Equine recurrent uveitis with PCR detection of *Leptospira* spp. in aqueous humor

Rebecca Egan, Davor Ojkic

Animal Health Laboratory, University of Guelph, Guelph, ON

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Equine recurrent uveitis (ERU), also known as periodic ophthalmia or moon blindness, is the most common cause of blindness in horses. Clinically, it presents as recurrent episodes of uveitis at random intervals and with increasing severity, and the Appaloosa has a known breed predisposition. The exact etiology and pathogenesis of this condition has not been fully elucidated, although there is evidence to suggest that leptospiral infection is a common inciting factor in this disease. It has also been suggested that an immune-mediated reaction may trigger autoimmune disease due to cross-reactivity between leptospiral antigens and endogenous ocular antigens. In one study by Faber et al., PCR testing and culture were performed on samples of aqueous humor from globes with a clinical diagnosis of ERU. It was reported that 21 of the 30 samples had PCR detection of *Leptospira* DNA in the aqueous humor, and culture of 6 of 21 samples isolated leptospires. Interestingly, 1 of the globes sampled from the 16 control horses with no current or historical evidence of uveitis was also positive by PCR. Serum was collected from these horses to evaluate for the presence of antibodies against five leptospira serovars, and it was found that the serologic results did not correlate well with the detection of *Leptospira* DNA or isolation of organisms from the aqueous humor. There were two aqueous samples that were positive by PCR and culture with negative serology, and none of the cultured isolates were serologically reactive with the serovars used in the microagglutination test (MAT), and this led the authors to speculate that these results might reflect infection with a different leptospiral serovar.

Between 2013 and 2022, a total of 391 equine samples were submitted for MAT to the Animal Health Laboratory for various reasons (**Table 1**). Among 173 samples with available history, 76.9% were from horses with ophthalmic issues (e.g., uveitis, cataract, blindness). All samples were tested for the presence of agglutinating antibodies against 7 *Leptospira interrogans* serovars: *L.* Autumnalis, *L.* Bratislava, *L.* Canicola, *L.* Grippotyphosa, *L.* Hardjo, *L.* Icterohaemorrhagiae, and *L.* Pomona. The positivity rate (titer of 100 or higher for at least one serovar) was 82.7% for samples with ophthalmic history. Among positive samples, 39.9% had antibody titers of 800 or higher. Samples submitted for other reasons had a comparable positivity rate of 76.9%; however, the occurrence of titers of 800 or higher was at a much lower rate of 10%. MAT may not be suitable for specifically identifying the infecting serovar because cross-reactivity may occur due to previous exposure and the characteristics of the test; however, in 110 submissions in which the single highest titer was determined, the most frequent were *L.* Pomona (33), *L.* Autumnalis (31) and *L.* Bratislava (28).

The Animal Health Laboratory recently received an enucleated eye from a 5-year-old female Warmblood horse. The patient had been assessed by an ophthalmologist and a clinical diagnosis of ERU was made. A blood sample had been submitted for *Leptospira* MAT at another laboratory, and results were negative; however, a false negative result is possible, if *Leptospira* spp. antigen of the infecting serovar was not present in the test panel. The severe panuveitis and band keratopathy in this horse did not improve with treatment, conferring a guarded long-term prognosis for vision in this eye; therefore, enucleation was elected.

Table 1: Summary of equine serum MAT testing, 2013-2022.

HISTORY	MAT Negative	MAT POSITIVE
Miscellaneous	6	18
Not given*	33	133
Ophthalmic	30	143
Renal	1	8
Reproductive	5	14
	75	**316**

*Samples with unknown history were not included in calculations

The enucleated globe was submitted to the AHL in formalin, and upon arrival at the lab, a sample of aqueous humor was collected via needle aspiration. Gross examination revealed a distinct localized region of corneal opacity at the medial aspect of the cornea, consistent with the clinically diagnosed keratopathy. Dissection of the globe identified a focally adherent inflammatory membrane along the posterior aspect of the ciliary body. Microscopic examination confirmed the presence of lesions typical of ERU, including lymphoplasmacytic inflammation in the ciliary body stroma, proteinaceous membrane with inflammatory cells along the posterior aspect of ciliary body processes (**Fig. 1**), and hypereosinophilic linear inclusions in the cytoplasm of non-pigmented ciliary body epithelium (**Fig. 2**). Immunohistochemistry performed on a section of the globe with ciliary body did not detect any immunoreactivity for leptospiral antigen. The aqueous humor was submitted for real-time PCR testing which detected the presence of *Leptospira* spp., and sequencing was applied in an attempt to identify the serovar. Based on partial SecY gene analysis, the sample was determined to be 99.5% similar to *L.* Grippotyphosa and 99.7% to *L.* Mozdok, and was thus not sufficient to confirm the exact identity of the serovar, as *Leptospira* genotyping results usually attain a 100% match or sequence homology. *AHL*

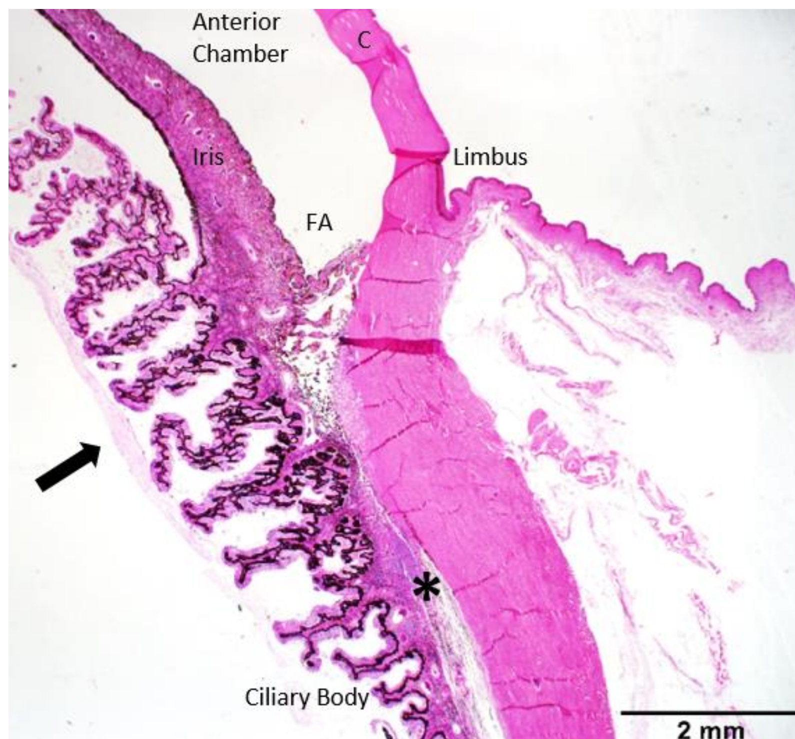

Figure 1. Microscopic section of the eye (H&E, 1.25x) demonstrating lymphoplasmacytic inflammation in the ciliary body stroma (*) and proteinaceous membrane with inflammatory cells along the posterior aspect of ciliary body processes (arrow). Cornea= C, filtration angle= FA.

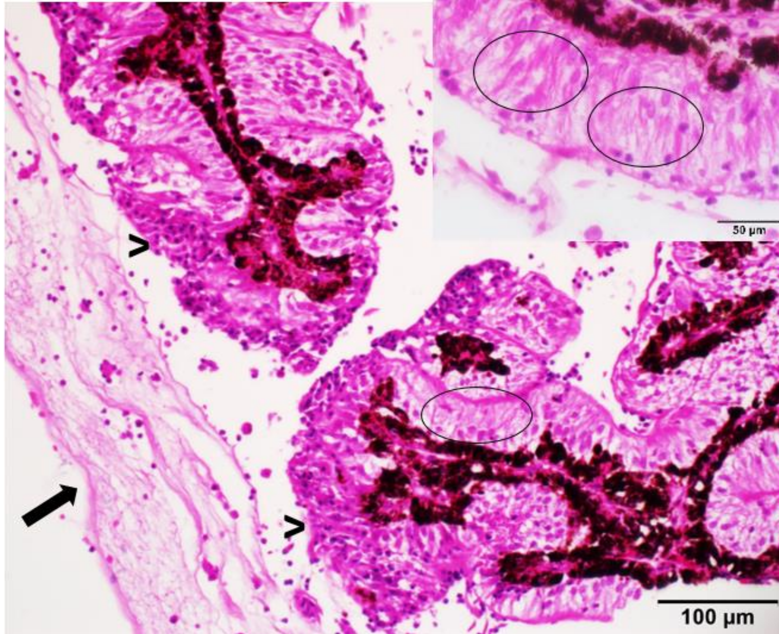

Figure 2. Microscopic section of eye (H&E, 20x) demonstrating lymphoplasmacytic inflammation within the epithelium ciliary body epithelium (>) and proteinaceous membrane with inflammatory cells along the posterior aspect (arrow). Inset (H&E, 40x): Hypereosinophilic linear inclusions in the cytoplasm of non-pigmented ciliary body epithelium.

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Platynosomum fastosum reported in a cat in Ontario

Emily Rätsep, Jacob Avula, Lindsay Ormesher

Animal Health Laboratory, University of Guelph, Kemptville, ON (Rätsep) and Guelph, ON (Avula); Mississauga Oakville Veterinary Emergency and Specialty Hospital, Mississauga, ON (Ormesher)

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In fall of 2022, samples of bile from the bile duct and gall bladder were received from a cat with history of having been obtained from Barbados. Vomiting was noted in Barbados in May 2022. Serum biochemistry performed June 2022 revealed mild hyperglobulinemia of 5.2 g/dL (RR: 2.8-5.1 g/dL), mild elevation in ALT of 295 U/L (RR: 12-130 U/L), and mild elevation in GGT enzyme activity of 7 U/L (RR: 0-4 U/L). A progressive hepatic enzymopathy was noted on subsequent visits, and abdominal ultrasound identified multiple cystic structures within the liver. The cat arrived in Canada and was presented to the internal medicine department at MOVEH in August 2022.

The cat was small in stature with a firm cranial abdomen, but no distinct organomegaly or masses. On ultrasound, heterogenous hepatic parenchyma was noted, and the previously-identified cystic structures were traced and confirmed as distension of all aspects of the biliary system. Persistent elevation in ALT enzyme activity at 406 U/L (RR: 27 - 158 U/L) and a novel, mild elevation in AST enzyme activity of 125 U/L (RR:16 - 67 U/L) was noted. Total and conjugated bilirubin were within normal limits. Functional liver parameters including glucose, urea, albumin and cholesterol were normal.

Based on the history and abdominal ultrasound findings, infection with *Platynosomum* spp. was suspected. Cholecystocentesis performed under sedation proceeded with separate aspirations of the gall bladder and the proximal bile duct. Bile samples were submitted to the Animal Health Laboratory for centrifugal sedimentation which revealed moderate numbers of oval, golden brown, thick-shelled operculated eggs with miracidia (33.0 µm x 42.0 µm), consistent with *Platysonosum fastosum* (**Figs. 1, 2**). An additional sample submitted to IDEXX confirmed many (>30) *Platynosomum concinnum (fastosum)* ova.

Resolution of the hepatic enzymopathy was noted following commencement of therapy. On follow-up examination, it was considered that based on the prepatent period of the parasite, immature flukes that were not eliminated with the first treatment may have matured into adults; therefore, treatment was repeated. It is uncertain if the observed ultrasound changes, considered secondary to injury and fibrosis induced by the flukes, would be permanent. A repeat cholecystocentesis was performed in November 2022, and no liver fluke ova or other parasites were identified.

Platynosomum fastosum is a small liver fluke from the family Dicrocoeliidae that is found in biliary ducts and gall bladders of cats, though ectopic sites have been reported, including small intestines, lung and pancreas. This parasite is generally found in tropical or subtropical areas, but has been reported world-wide. The lifecycle is still not fully understood, but generally involves snail intermediate hosts (*Sublima octona*) with terrestrial isopods, lizards, toads and geckos acting as paratenic hosts. Cats acquire the infection by ingesting infected lizards containing the encysted infective stage metacercariae, hence the commonly-recognized name of “lizard poisoning” in cats. The metacercariae excyst in the small intestine of the cat, and travel through the duodenal papillae to the common bile duct, bile ducts and gall bladder where they mature into adult flukes. Eggs are discharged intermittently in the feces of the infected cat.

While infection is often asymptomatic and considered an incidental finding, obstructive hepatobiliary disease with hepatic failure can occur in some cats with a heavier burden. Clinical disease presents as jaundice, abdominal distension and weight loss. Anorexia, diarrhea, vomiting, depression, hair loss and slight fever have also been reported, together with biochemical changes of elevated hepatic enzymes and total bilirubin vales. Parasitic infection of bile ducts can result in cholangitis/cholangiohepatitis, characterized by hyperplasia of the epithelium, periductal fibrosis, inflammation, and occasionally, progressive obstruction due to the presence of adult flukes. These lesions can present as feline cystic hepatic disease.

Diagnosis is based on history, clinical findings and detection of the adults or eggs in stool or bile, on ultrasound or during postmortem. Shedding of eggs in stool is intermittent, and eggs may not appear if infection results in biliary obstruction. Identification of the trematodes and/or eggs in bile aspirates is considered a more effective method of diagnosis. *P. fastosum* ova can easily be differentiated from the more common *Paragonimus kellicotti* lung fluke eggs in fecal samples, as the latter are much larger in size, have a thickened ridge on the shell beside the operculum, and contain an embryonic cell mass.

Considering these findings, platynosomiasis should remain a potential differential diagnosis for felids with hepato-biliary dysfunction in Canada, especially in those with a history of travel to or from tropical and subtropical locations. *AHL*

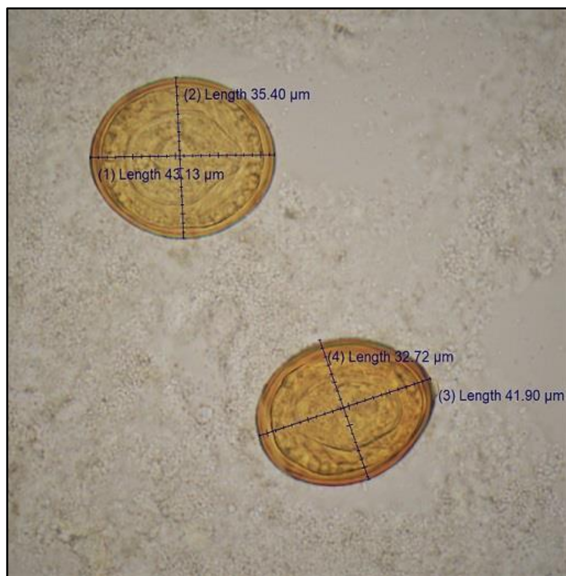

Figure 1. *Platynosomum fastosum* eggs obtained from centrifugal sedimentation of bile with measurements.

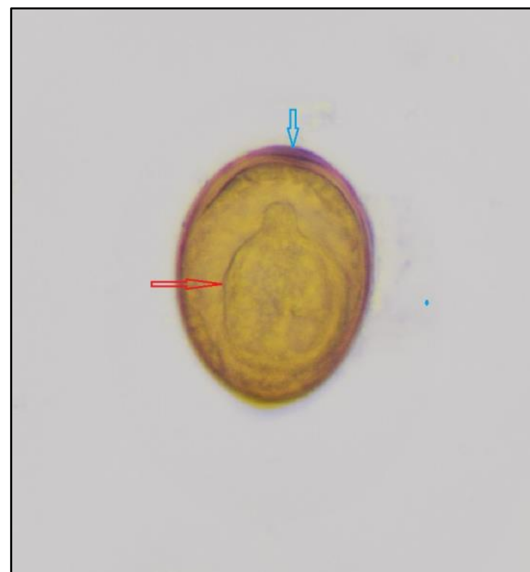

Figure 2. Fluke eggs showing operculum (blue arrow) and miracidium (red arrow).

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