Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

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Update from the Director

The view from the Director's office

The recent COVID-19 Omicron wave certainly caused some challenges in lab operations during January and February. In some ways, this spike was the most difficult to manage due to the rise in post-holiday infections, the marked communicability of this particular variant, and the isolation requirements for families. Although some lab sections were short-staffed for brief periods, we were able to maintain satisfactory turnaround times. We thank our clients for their patience and understanding during this time.

Our recent focus has been on preparing the lab for a possible outbreak of highly pathogenic (now called notifiable) avian influenza. H5N1 influenza has been circulating in Europe and other countries for several years and is spread predominantly by wild waterfowl. This infection causes high mortality in poultry, and outbreaks are therefore subject to OIE notification, quarantine and eradication. In December 2021, H5N1 was diagnosed in wild birds in both Canada and the USA, and is now infecting commercial poultry and backyard flocks in both countries. It is theorized that infected birds from Europe reached North America and are now traveling the Atlantic flyway: https://www.biorxiv.org/content/10.1101/2022.01.13.476155v1. As a member of CAHSN (Canadian Animal Health Surveillance Network), AHL supports the CFIA response by testing negative flocks for movement through the control zones.

This issue of the AHL newsletter contains the second section of our antibiotic sensitivity LabNote 64 that describes how antibiotic sensitivities are performed at the lab, and how results should be interpreted to support prudent use of this class of antimicrobial drugs. This is also the annual issue in which we provide a summary of zoonotic pathogen test results, and a comprehensive list of new lab tests and test panels developed over the past year.

We hope that the arrival of spring will accompany the return of a more normal life than we have experienced over the past two years. We wish you all good health and renewed optimism.

Maria Spinato, Director
Animal Health Laboratory, University of Guelph, Guelph, ON.

AHL New Tests Developed in 2021

Jim Fairles

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

AHL Newsletter 2022;26(1):3.

TEST METHOD	CODE	SPECIES
Bacterial culture, *Campylobacter fetus* subsp. *venerealis*	cultcfv	Bovine
Bovine adenovirus sequencing	badvseq	Bovine, Ovine
Bovine astrovirus - PCR	boastpc	Bovine
Bovine comprehensive respiratory panel	brsppnl	Bovine
Bovine viral diarrhea virus/Bovine adenovirus/Bovine coronavirus - triplexPCR	bvdadco	Bovine, Ovine
Chicken proventricular necrosis virus - PCR	cpnvpcr	Avian, Chicken
Cryptosporidium species PCR	crypto	All species
Equine adult diarrhea PCR panel	adpcrp	Equine
Equine encephalitis virus (EEEV)/West Nile virus (WNV) – duplex PCR	eeewnv	Avian, Equine
Equine foal diarrhea PCR panel	fdpcrp	Equine
Fish infectious pancreatic necrosis virus (IPNV) - PCR	ipnv	Other
Hemorrhagic enteritis virus, turkey - ELISA	heveli	Avian, Turkey
Infectious bronchitis virus/Infectious laryngotracheitis virus - duplex PCR	ibviltv	Avian, Chicken
Infectious bursal disease virus/Chicken anemia virus - duplex PCR	ibdvcav	Avian, Chicken
Malignant catarrhal fever PCR (ovine herpesvirus-2)	mcfpcr	Bovine, Caprine, Ovine, Porcine
Porcine circovirus 1,2,3 - triplex PCR	pcv123	Porcine
Porcine circovirus type 3 - Sequencing	pcv3seq	Porcine
Salmonella Dublin - PCR	sdpcr	Bovine
Serpentovirus (reptile nidovirus) RT-qPCR	serppcr	Other
Whole genome sequencing	wgs bac	All species

Final submissions for OAHN swine small herd postmortem project due by March 31, 2022

Please note that the **final date for acceptance of eligible submissions for the OAHN Swine Small Herd Postmortem Project will be March 31, 2022**. We thank all of the veterinarians who have submitted cases that support the expanded surveillance of small swine herds throughout Ontario.

OAHN Update – March 2022

Mike Deane, Tanya Rossi

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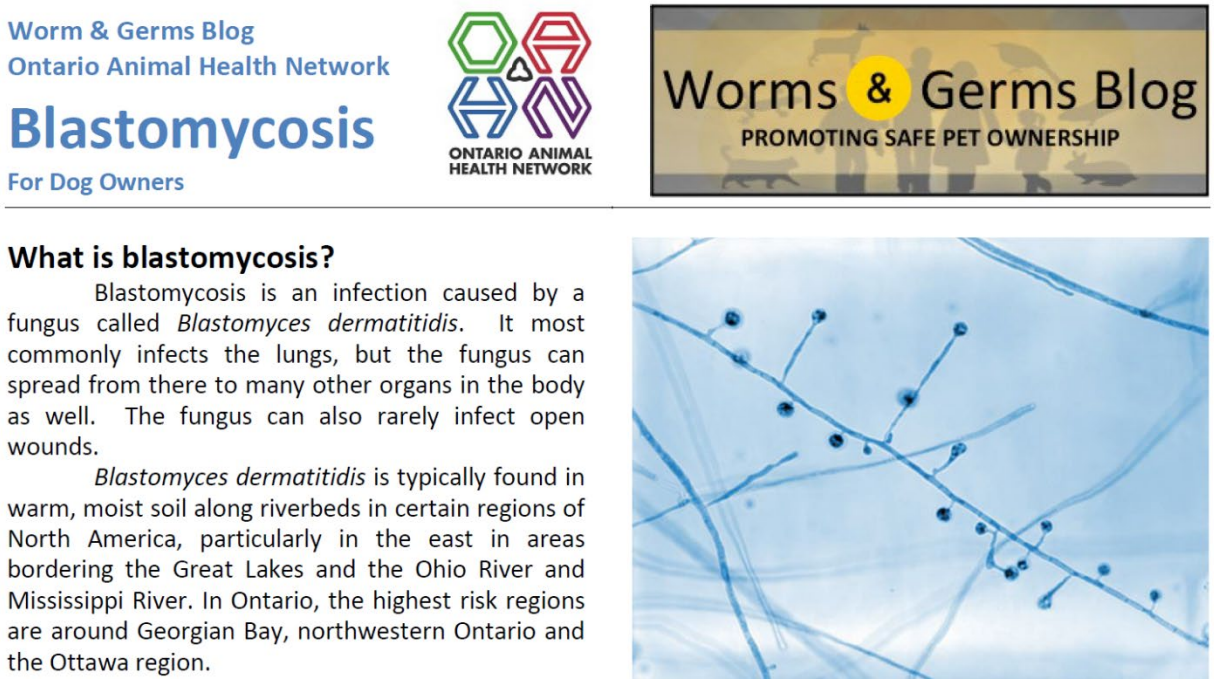
This fall and winter, OAHN has been very busy creating new animal health resources, and finishing up many research projects. In addition, we held our quarterly species-specific meetings and created the resultant veterinary and owner/producer reports which reflect what is being seen in veterinary practice, labs, and abattoirs throughout Ontario. To view the reports, go to OAHN.ca and navigate to the species you are interested in.



New Resources

- Infosheet: Blastomycosis for Dog Owners
- Aquaculture Biosecurity Fact Sheet
- OAHN Bovine Network Quarterly Update January 2022
- Infographic: Avian Influenza Information for Backyard Flock Owners

Completed Projects

- OAHN Bee Network Project: Mapping the population density of managed honey bee colonies in Ontario
- OAHN Equine Network Project: Investigation of a respiratory disease outbreak in the Ontario harness industry and the economic impact on industry participants
- OAHN Small Ruminant Research Project: Comparison of maedi visna serological tests including the VMRD ELISA

New Reports

- Surveillance: Q3 Animal Health Laboratory Data
- Diagnostics in Respiratory Disease Outbreaks
- Spotlight on MAP
- Herd Profile – Disease Monitoring
- Assessing Johne's Results in DairyComp

- Infectious Bronchitis Virus (IBV) and Infectious Bursal Disease Virus (IBDV) strains – By Commodity
- Poultry Veterinary Survey Highlights – Q4

- Disease Discussion
- Laboratory Diagnostic Reports
- Slaughter Statistics
- Topics of Interest

- Pituitary Pars Intermedia Dysfunction
- EEE linked to a bird?
- OAHN Q3 survey: Key results
- Equine asthma chart
- Equine Research

- OAHN winter survey and lab data: Key results
- Lung fluke cluster
- Rabies recap 2021
- Blastomycosis cases, owner infosheet
- Firstline app: CVMA AMU guidelines

Selected zoonotic pathogens and diseases from Ontario identified at the AHL in 2021

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AHL Newsletter 2022;26(1):6.

As the current pandemic highlights, emerging, and re-emerging human infectious diseases often originate or persist in animal reservoirs. The AHL plays an important role in surveillance of zoonotic pathogens in many domestic and wild animals by annually reporting case counts (**Tables 1, 2**).
The percentage of animals identified as positive for leptospirosis was roughly unchanged in 2021 in all species except equines, where there was a moderate decrease in cases. The total number of submissions tested was approximately the same as 2020 in all species. These data do not take into account vaccination status, as many species may have previously been vaccinated for leptospirosis.

The discrepancy between *Brucella canis* cases between 2020 and 2021 (**Table 1**) can be explain by a change in tests used (in 2020 only 2ME-RSAT positive cases were included). There was a moderate increase in MRSP, and all *Strep* spp. included in this report. Equine eastern encephalitis virus cases also increased, although overall numbers remain low. These data are case counts from AHL submissions and subject to laboratory submission biases. Therefore, results may not represent the whole picture of zoonotic diseases in Ontario and should not be used to calculate population prevalence estimates. Monitoring programs are not included. *AHL*

Table 1. Number of cases with selected zoonotic pathogens isolated and/or identified at the AHL in 2021.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2021	2020
Ascarids (incl *T. canis, T. cati, T. leonina, Baylisascaris* sp.)			6			71		18		5	100	**93**
Blastomyces dermatitidis								5			5	**7**
Bordetella bronchiseptica	2	47	5	1				3		5	63	**51**
***Borrelia burgdorferi* (Lyme disease), serology**			25					11			36	**34**
***Brucella* sp. (non-*abortus*)**								131			131	**14**
Campylobacter coli/ jejuni/ fetus* subsp. *fetus	2			4				3			9	**7**
***Chlamydia* sp.**		2		9	11						22	**19**
Clostridium difficile			1		1						2	**3**
***Coxiella burnetii* (Q fever)**	9			26	21						56	**45**
***Cryptococcus* sp.**											0	**0**

***Cryptosporidium* sp.**	178			2	7				3	11	201	**239**
Eastern equine encephalitis virus			8								8	**4**
Echinococcus multilocularis								2			2	**2**
***Giardia* sp.**	8							22	1		31	**35**
Listeria monocytogenes	8	1		5	4			1	2		21	**25**
Methicillin-resistant *Staph aureus* (MRSA)		1	1					2		1	5	**6**
Methicillin-resistant *S. pseudintermedius* (MRSP)								87	2	1	90	**62**
Rabies virus										4	4	**5**
Salmonella enterica	56	78	4	4		29	54	2		18	245	**245**
Streptococcus suis	1	176	1	1		6	1				186	**148**
Streptococcus equisimilis	1	45	14		1			3			64	**48**
Streptococcus zooepidemicus	2	3	155		2			4	1	1	168	**140**
***Toxoplasma* sp.**				13	3				2		18	**15**
Verotoxigenic *E. coli* (VTEC)	3	3									6	**6**
West Nile virus			3							11	14	**27**
Yersinia enterocolitica	1	1						2			4	**5**
Total											1491	**1285**

Table 2. *Leptospira* spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL in 2021.

Leptospira spp. serovar	**Bovine**	**Swine**	**Equine**	**Canine**	**Other**
L. autumnalis	26	4	25	79	1
L. bratislava	19	4	21	48	1
L .canicola	26	2	13	46	1
L. grippotyphosa	9	1	4	43	1
L. hardjo	32	1	7	17	1
L. icterohaemorrhagiae	30	4	15	65	1
L. pomona	34	4	13	68	1
IHC or PCR-positive	0	0	1	7	0
Positive/tested cases	**54/173**	**4/28**	**35/65**	**115/199**	**1/2**

Antimicrobial susceptibility testing: Part 2 - Bacterial organisms

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AHL Newsletter 2022;26(1):8.

When performing **a**ntimicrobial **s**usceptibility **t**esting (AST), all diagnostic laboratories in North America are expected to follow the standards and guidelines established by Clinical Laboratory Standard Institute (CLSI). The goal of this institute is to promote accurate and reproducible AST results, as well as appropriate reporting and interpretation by standardizing all aspects of AST. A sub-committee on veterinary antimicrobial susceptibility testing (VAST) establishes guidelines for veterinary laboratories that include: testing methods, bacterial density, media and drug types, drug dilutions, incubation times, quality control (QC) requirements, and - most importantly - interpretative criteria.

Some of these recommendations are being reviewed in a series on antimicrobial susceptibility testing in AHL newsletters, beginning with the article on test methods published in December 2021. The second article in our series, presented here, pertains to selection of bacterial organisms for antimicrobial susceptibility testing. The full document is available in LabNote 64: https://www.uoguelph.ca/ahl/ahl-labnote-64-antimicrobial-susceptibility-testing-ast

1. It is acceptable to proceed with empirical treatment for bacterial species that have a predictable susceptibility with low level or no known resistance to certain drugs.

    a) Penicillin is the recommended treatment choice for beta haemolytic streptococci including *Streptococcus equi* subsp. *equi*, *S. zooepidemicus*, and *S. canis*. It is also a preferred treatment option for *Trueperella pyogenes*.

    b) It is recommended to test a bacterial organism that contributes to an infectious process requiring treatment if its susceptibility cannot be reliably predicted based on bacterial identification.

    c) AST is most often indicated for bacterial species known to have the ability to develop resistance to commonly used antimicrobial agents (i.e., *E. coli*, *Staphylococcus* spp., *Pseudomonas aeruginosa*).

    d) Both AST methods have been developed for routine testing of fast-growing aerobic bacteria and are modified for testing of some fastidious bacteria such as *Actinobacillus pleuropneumoniae*, *Mannheimia haemolytica* and *Histophilus somni.*

2. It is discouraged to perform AST on normal bacterial flora such as coagulase negative *Staphylococcus* spp. from skin samples.

3. CLSI guidelines are not available for all bacterial species. Breakpoints are not established for certain veterinary pathogens because they may be difficult to grow under standard testing conditions (i.e., *Glaesserella (Haemophilus) parasuis*), they require a higher biosafety level than may be available in veterinary diagnostic labs (i.e., *Brucella* spp.), or if they are newly discovered. *AHL*

Cache Valley virus abortions in goat kids

Amanda Mansz, Emily zur Linden, Heather Murray, Heindrich N. Snyman

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AHL Newsletter 2022;26(1):9

In early December 2021, two separate and distinct dairy goat herds (one in Eastern Ontario and one in Southwestern Ontario) were entering their kidding season when they suddenly started to experience significant reproductive losses. In both cases, aborted fetuses exhibited various combinations of fetal malformations, including, limb contracture/arthrogryposis, scoliosis/kyphosis/lordosis, cerebellar hypoplasia, and hydrocephalus **(Fig. 1)** .

The first herd (n = 25 with 7 pregnant does) consisted of 2nd and 3rd lactation does with approximately half of the animals kidding affected fetuses. The second herd (n = 225 with 85 pregnant does) contained does of various lactation stages with 16/120 kids (13%) being affected, and four does lost as a result of associated birthing complications. The first herd had no previously reported reproductive losses, while the second herd had reported similar fetal malformations 5 years previously.

Following postmortem and microscopic evaluation, samples of placenta, fetal tissues and fetal thoracic fluid from both cases were independently sent to Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for detection of Cache Valley virus (CVV). PCR testing for CVV on placental and/or fetal tissues was negative, while the virus neutralization (VN) assay on fetal thoracic cavity fluid was positive in both cases. Tissues from both cases also tested negative for both *Coxiella burnetti* and *Chlamydia abortus* by PCR testing. *Toxoplasma gondii* PCR on samples from the first herd was also negative, and no bacterial pathogens were isolated in that case.

In sheep and goats, CVV infection can cause infertility, abortion, stillbirths and congenital anomalies with the latter occurring specifically when infection occurs between 28 - 48 days of gestation. CVV is a mosquito-borne bunyavirus that is considered endemic across much of North America, including Ontario. Transmission occurs predominately through mosquitoes (various *Aedes* spp.), but biting culicoid midges are also implicated. In Ontario, the population of infected mosquitos reaches its peak in late summer/early fall, resulting in abortions/stillbirths during December and January. Does are typically asymptomatic with rapid clearance of the virus following infection, and therefore, virus is often not present within tissues at the time of abortion.

The rapid clearance of the virus is the reason for a high likelihood of a negative PCR test result. Although fetal fluid is not the specifically validated sample type for serological antibody testing, it is often the only sample available for testing in aborted fetuses. The positive antibody titre on fetal thoracic fluid in this case is indicative of historic infection with CVV and supports CVV infection as the cause of these abortions. Performing titres on the ewes/does may also be helpful to confirm exposure in a flock.

Similar congenital malformations can also be associated with other infections (e.g., border disease, bluetongue virus, bovine viral diarrhea virus), inherited genetic defects (e.g., ovine hereditary chondrodysplasia/spider lamb syndrome), and ingestion of teratogenic plants (e.g., *Lupinus* spp.).

Therefore, submission of the whole fetus and placenta for diagnostic evaluation forms an important component in any investigation of fetal losses in a flock.

Given the endemicity of the virus in Ontario, CVV-associated abortions are sporadically diagnosed in sheep at the AHL. However, to date, these are the only two cases where CVV has been confirmed as the cause of abortion in any goat population tested at the AHL. *AHL*

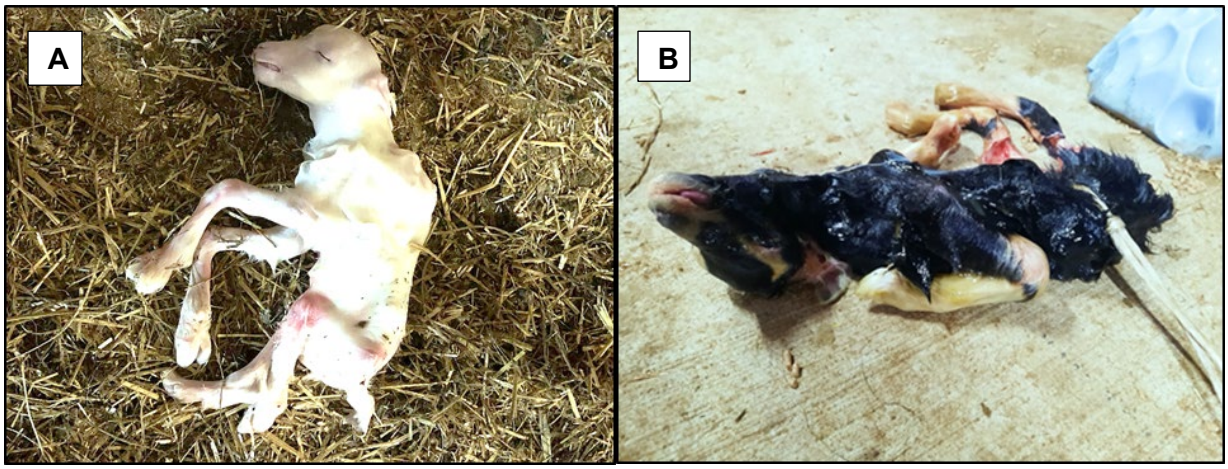

Figure 1. Aborted goat fetuses following CVV infection. A. The fetus has prominent thoracic kyphosis with a shortened vertebral column and variable arthrogryposis of all four limbs (photo courtesy of Dr. Heather Murray; Dundas Veterinary Services). B. The entire length of the vertebral column of this fetus is markedly twisted (scoliosis) and all four limbs contain flexural contracture (photo courtesy of Dr. Emily zur Linden; Metzger Veterinary Services).

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1. LWaddell L, et al. Cache Valley virus: A scoping review of the global evidence. Zoonoses Public Health. 2019;66:739–758.
2. Rodrigues Hoffmann A, et al. Ovine fetal immune response to Cache Valley virus infection. Journal of Virology, 2013; 87:5586-5592.
3. Menzies PI. Abortion in Sheep: Diagnosis and Control. In: Youngquist RA, Threlfall WR, eds. Current Therapy in Large Animal Theriogenology. 2nd ed. St Louis, MO: Saunders Elsevier Inc, 2007:667-680.

Update on ruminant testing at AHL

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AHL Newsletter 2022;26(1):10

Recently, AHL Virology has realigned ruminant testing and added some new tests:

1. Bovine viral diarrhea virus/bovine adenovirus/bovine coronavirus – test code: bvdadco

This triplex test combines bovine viral diarrhea virus (BVDV) PCR, bovine coronavirus (BCoV) PCR, and a new test, bovine adenovirus (BAdV) PCR. The BAdV PCR cross-reacts with multiple BAdV types and will detect BAdV 1,2,3,4,5,6,7 and 8.

> **Note 1:** The triplex BVDV/BAdV/BCoV PCR will now be routinely used when BVDV testing is requested. If BVDV PCR only is required, e.g., for export or surveillance purposes, please indicate on the submission form that the purpose of BVDV testing is export/surveillance and we will test samples with a BVDV-only PCR test.
>
> **Note 2:** The triplex BVDV/BAdV/BCoV PCR can be used for investigation of respiratory and/or enteric diseases.
>
> **Note 3:** When BVDV/BAdV/BCoV PCR yields a positive BVDV result, BVDV typing can be done at an additional cost, upon request.

2. Bovine adenovirus sequencing – test code: badvseq
When BVDV/BAdV/BCoV PCR yields a positive BAdV result, the sample can be further genotyped/classified by adenovirus hexon gene sequencing at an additional cost, if desired.

3. Malignant catarrhal fever (MCF – ovine herpesvirus-2) PCR – test code: mcfpcr
Samples with a constellation of clinical and histologic findings that were consistent with malignant catarrhal fever were used for PCR validation. MCF PCR results and histologic findings were in 100% agreement.

The **Bovine rotavirus/corona virus PCR** remains unchanged **(Rotavirus/coronavirus, bovine/equine - PCR. Includes: coronavirus - PCR, rotavirus Group A and B – PCR – test code: rocopcr).** Because rotavirus infection is not significant in respiratory cases, this test will now only be used in enteric case testing including the Bovine enteric panel (**Bovine neonatal enteric panel – test code: bentpnl**).

The **Bovine comprehensive respiratory panel (Comprehensive bovine respiratory disease panel – test code: brsppnl)** has now changed to reflect the testing changes described above. It will now include:

- Bovine viral diarrhea virus/bovine adenovirus/bovine coronavirus PCR – bvdadco
- Bovine respiratory virus panel PCR (BoHV-1/IBR, BPiV-3, BRSV) - brvp3
- Bacterial culture, food/fiber producing animals (other than swine) – cultf
- *Mycoplasma bovis* PCR – mbpcr

Sampling and submission guidelines remain the same: 2 deep nasopharyngeal samples need to be included for the test – 1 for PCR (swab in virus transport media or red top tube with 0.5 mL of saline) and 1 for culture (bacterial culture swab). If pooling is requested, the panel will be split into the individual tests, as pooling can be done only for the virology PCR tests. Pooling is not recommended for bacterial culture since a nasopharyngeal swab is considered a highly contaminated sample. There is now one less test in this panel, and the price has been reduced accordingly.

Pooling is available for all PCR testing. Please note: when pooling is requested, a pooling charge is added per sample to cover the labour and consumables needed for pooling.

The ruminant submission form is being updated to reflect these changes. *AHL*

SWINE

CanSpotASF reminder

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AHL Newsletter 2022;26(1):12.

The Animal Health Laboratory continues to test appropriate swine cases under the CanSpotASF surveillance program. Eligible cases are those for which African swine fever is not a differential diagnosis. If ASF is considered as a differential diagnosis, then CFIA must be contacted immediately.

To qualify for CanSpotASF surveillance testing, cases must have herd location information available (PID or physical address), and submit fresh spleen, tonsil, kidney, lymph node, terminal ileum, or serum for testing. Only these samples are appropriate for the CanSpotASF surveillance program.

Please note – the program has been updated so that pathology and non-pathology cases can be included in the program as long as they meet the presentations eligible for CanSpotASF testing. **Practitioners may now submit directly as part of a case without pathology testing (postmortem and/or histopathology) required**. Please note all criteria that apply in the history.

To facilitate the success of this ASF surveillance, veterinarians should:

- **submit fresh spleen, tonsil, kidney, lymph node, terminal ileum, or serum**
- include a thorough clinical history with each case
- **ensure that the herd PID or physical address is included**
- for pathology cases - please respond to pathologists' requests for permission for surveillance testing if blanket approval has not been provided ahead of time

Clinicopathological presentations eligible for CanSpotASF testing:

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

Please contact Jim Fairles (jfairles@uoguelph.ca) or your case pathologist with any questions about the CanSpotASF program and the submission process.

Thank you for contributing to enhanced ASF surveillance! *AHL*

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

Marek's disease

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AHL Newsletter 2022;26(1):13.

Marek's disease virus (MDV) is a cell-associated alphaherpesvirus belonging to the genus *Mardivirus*. The classification of this virus can be confusing as it has evolved over time (**Table 1**), but the original terminology is still in use. **In general, MDV refers to *Gallid herpesvirus 2* (serotype 1, prototype virus).** Tumour production is associated only with serotype 1 viruses that vary widely in virulence. Serotype 1 viruses are further divided into pathotypes based on virulence (**Table 1**). Virtually all chickens are susceptible to MDV infection and tumor development. Quail, turkeys, partridges, pheasants, and some species of ducks and geese, are also susceptible to infection and disease.

Table 1. Marek's Disease virus classification.

Previous Classification: Serotypes	**Current Classification:** Species Family: *Herpesviridae* Subfamily: *Alphaherpesvirinae* Genus: *Mardivirus*	**Pathotype**
Serotype 1 (Oncogenic MDV & attenuated strains)	*Gallid alpha herpesvirus 2* (GaHV-2)	mild (mMDV) virulent (vMDV) very virulent (vvMDV) very virulent plus (vv+MDV)
Serotype 2 (Nononcogenic MDV, chickens)	*Gallid alpha herpesvirus 3* (GaHV-3)	
Serotype 3 (Nononcogenic turkey herpesvirus, HVT)	*Meleagrid alpha herpesvirus 1* (MeHV-1)	

Marek's disease virus is ubiquitous. Feather follicle epithelial cells in feathers and dander are the main source of environmental contamination. The virus can remain infective for 4-8 months at room temperature and years at 4C. A variety of chemical disinfectants can inactivate the virus within a 10-minute application time.

Inhalation is the natural route of infection. MDV is transmitted by direct contact (residual house dust and dander, fomites, personnel) or indirect contact (aerosolized from adjacent houses) between chickens. Virus excretion begins approximately 2 weeks post infection and continues indefinitely with maximum shedding 3-5 weeks after initial infection. Once the virus is introduced into a chicken flock, infection spreads quickly from bird to bird, regardless of vaccination status or genetic resistance. Vertical transmission does not occur.

Marek's disease begins as an inflammatory condition that will either regress, become latent within 7-8

days post infection but still detectable in lymphoid organs and peripheral blood lymphocytes, or progress to development of lymphomas. Based on disease progression, clinical syndromes and lesions can be generally divided into non-neoplastic and neoplastic. Non-neoplastic syndromes that may occur in unvaccinated flocks include: lymphodegenerative syndromes (early mortality syndrome, cytolytic infection, immunodepression); CNS syndromes (transient paralysis, persistent neurologic disease of limbs or neck); and atherosclerosis.

Neoplastic syndromes can have non-specific clinical signs related to peripheral nerve dysfunction including crop dilation or gasping (i.e., vagus nerve involvement), incoordination, stilted gait, or unilateral paralysis with a characteristic clinical presentation of one leg forward, one leg back (**Fig. 1A**). Birds with lymphomas may have no clinical signs or may present with depression, weight loss, pallor, anorexia, diarrhea, torticollis, and/or unilateral or bilateral blindness, with or without 'gray eye' which is typified by a gray discolouration of the iris and an irregular pupil. Lymphomas can occur in any internal organ (**Fig. 1B**), as well as brain, nerves, eye and skin. Tumours can be nodular or cause diffuse enlargement of organs. Nodules are white or gray, firm, and smooth on cut surface. On histopathology, the tumours consist of mixed populations of neoplastic lymphoid cells and inflammatory cells that include lymphocytes (primarily T cells) and macrophages (**Fig. 1D**).

There is no effective or practical treatment for individual chickens or infected flocks. Control is a challenge due to ubiquitous virus, latent infection, continuous viral shedding, and long-term survival of virus in the environment. Vaccination, biosecurity, and genetic resistance are key components of control. Vaccines in order of increasing efficacy are: HVT (FC126), mixed HVT + serotype 2 MDV (SB1, 301B), and attenuated serotype 1 MDV (CVI988). Vaccines do NOT prevent infection but they do protect against early replication of virulent viruses in lymphoid organs, and reduce the level of latent infection. Vaccines can be given *in ovo* at day 18 of incubation, or at hatch (SQ or IM). At least 7 days are required to establish solid immunity after vaccination.

Increased cases in a flock can be caused by unvaccinated birds, improper vaccination, vaccine strain (weak strain of vaccine may not protect against very virulent strains), early exposure (before vaccinal immunity is established), stress (e.g., onset of lay), and immunosuppression due to other diseases and viral infections such as CAV, IBDV and reoviruses.

Diagnosis: Detecting virus alone is not sufficient to make a confirmatory diagnosis, unless this detection is associated with characteristic clinical signs and lesions including visceral tumours and peripheral nerve infiltrates.

Samples:
Histology: peripheral nerves (sciatic, brachial plexus, vagus), brain, tumours
PCR: a) Send-out test to Quebec:
Samples and collection: (shipping and handling within Canada: $50 CAD, each PCR: $40 CAD)
1) 1 mL EDTA blood - collect blood into a purple-top tube
2) tissues, feather tip - sterile, leak proof container
3) postmortem – lymphoid tissue (spleen, liver) or tumours

b) Send-out test to Poultry Diagnostic Research Center – PDRC, Georgia, USA: provides more options for typing of virus. Please contact AHL Specimen Reception for details and pricing. *AHL*

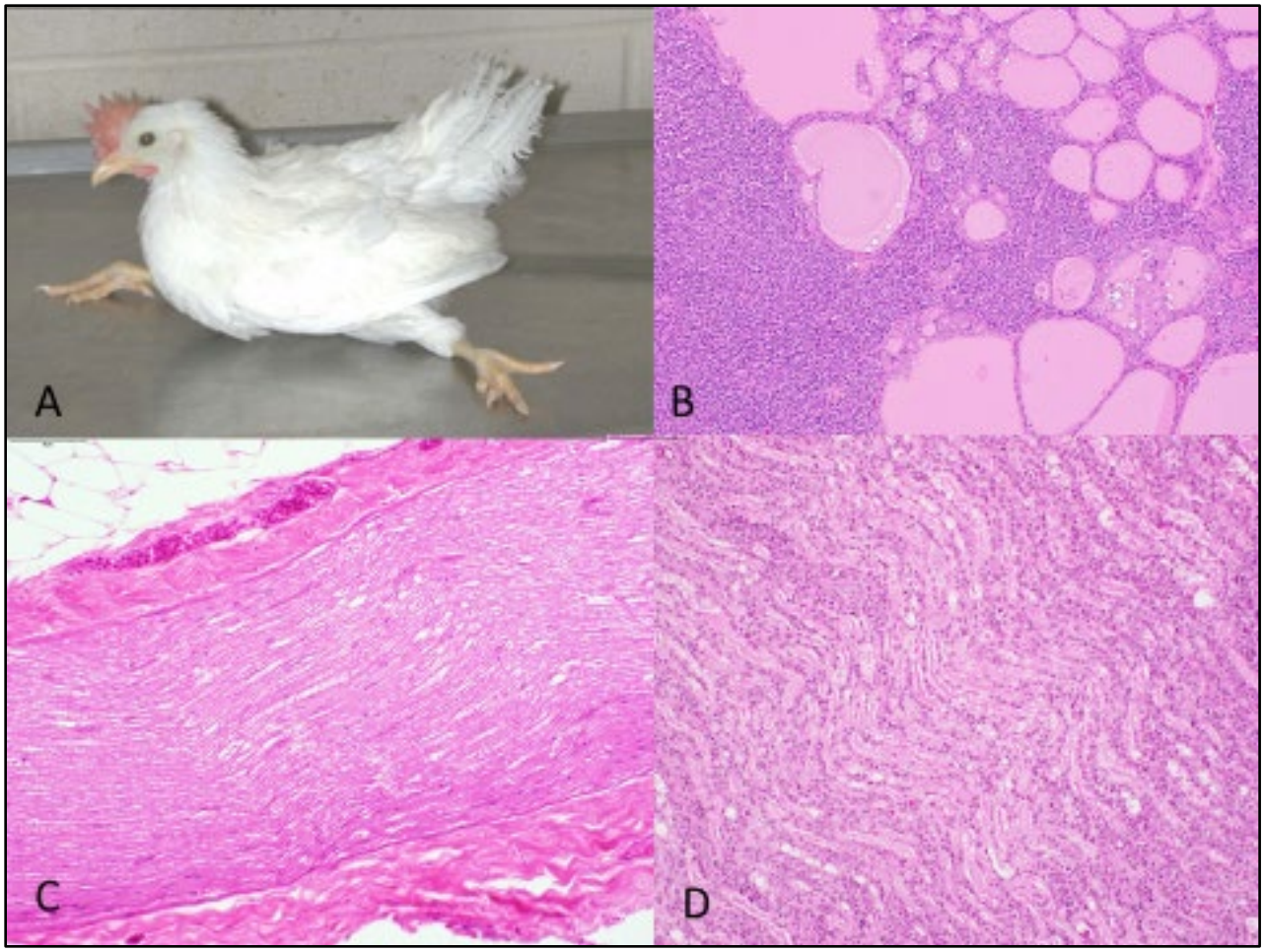

Figure 1. Clinical presentation and histologic lesions of MDV. (Photos B, C, D by E. Martin, H&E stain.)
A. Clinical presentation of one leg forward/one leg back (AAAP Marek's Disease Slide Set).
B. Thyroid lymphoma expanding space between follicles. C. Normal sciatic nerve. D. Severe peripheral neuritis, sciatic nerve. Extensive cellular infiltrates, most consistent with MDV.

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Infectious bursal disease virus and chicken anemia virus testing update

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Immunosuppression associated with viral infection is frequently suspected as an underlying cause or contributor in various aspects related to management of poultry health issues. Infection with many viruses can cause, or be involved in, immunosuppression, e.g., infection with Marek's disease virus (MDV), reticuloendotheliosis virus (REV), avian leukosis virus (ALV), chicken anemia virus (CAV), infectious bursal disease virus (IBDV), avian reovirus (ARV) and fowl adenovirus (FAdV). Viruses such as CAV and IBDV produce significant lesions in the primary lymphoid tissues (**Fig. 1**). The impact of some of these agents in modern poultry production systems has been minimized through vaccination (MDV) and/or eradication (REV, ALV). Despite intensive vaccination programs in both breeder and broiler flocks, IBDV infection has continued to evade control attempts. On the other hand, the impact of CAV infection appears to be less significant, and is better controlled by breeder vaccination and subsequent maternal antibody transfer to broiler birds. Still, synergism of IBDV and CAV co-infection has been reported, and can exacerbate the negative effects of these immunosuppressive viruses.

AHL previously had two separate PCR tests for the detection of IBDV and CAV. Recently, we investigated the frequency of IBDV and CAV co-infection by testing forty-eight samples (bursa of Fabricius) that were positive for IBDV by CAV PCR. Fourteen samples (29%) were also positive for CAV, and based on PCR Ct values, 79% (11 of 14) of these CAV-positive samples had a higher load of CAV than IBDV (**Table 1**).

As a consequence, AHL has combined IBDV and CAV PCR tests into a duplex PCR test to allow continuous detection/monitoring of IBDV/CAV co-infection. The duplex test is now routinely available and is offered for the same fee as a single PCR test (currently $36). Genotyping of samples positive for IBDV and/or CAV is available at an additional cost. *AHL*

Table 1. IBDV and CAV co-infection and viral load (Ct value) in the bursa of Fabricius

AHL #	IBDV Ct	CAV Ct
19-097240-0002	24.35	21.13
20-003254-0002	28.62	21.78
19-097244-0001	29.13	22.15
21-005178-0001	23.88	22.85
20-032450-0001	28.47	23.02
19-097237-0002	28.46	23.07
20-042526-0001	29.06	26.3
19-032291-0002	30.13	26.8
18-074672-0001	29.25	27.16
20-092542-0001	29.23	27.62
20-011787-0002	29.65	28.61
19-040302-0003	28.67	29.9
21-006401-0001	28.64	31.02
19-067017-0001	28.31	34.31

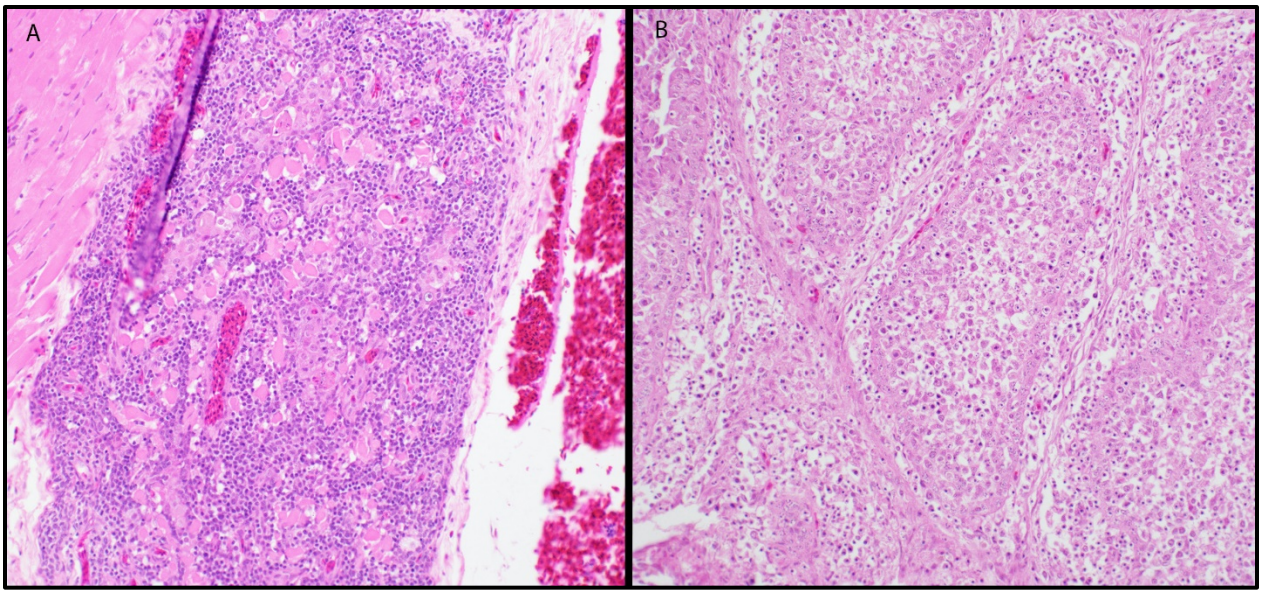

Figure 1. Histologic lesions caused by immunosuppressive viruses. **A**. Thymic atrophy in a 10-week-old broiler-breeder chicken associated with CAV infection (the flock was not vaccinated for CAV). **B.** Severe depletion of lymphocytes from the bursa of Fabricius of a 6-week-old layer chicken associated with IBDV infection. Vp2 gene sequencing indicated a field strain of IBDV. (H&E stain).

Fenthion poisoning in ravens

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Sick and dead ravens were found in proximity to a pile of fish offal, beef bones and coyote carcasses in Manitoba. The pile was suspected to be poisoned bait; therefore, local conservation officers proceeded to submit beef bones and five dead ravens to Veterinary Diagnostic Services, Manitoba Agriculture for diagnostic testing. On postmortem (PM) examination, the birds were all adults in good body condition. One raven had been shot and another had been scavenged and looked more autolyzed than the others. Only one bird had identifiable stomach contents, consisting of fish bones and scales. There were no other significant gross findings.

Considering multiple deaths and the lack of significant PM findings, toxicosis was suspected. To investigate exposure to pesticides, brains from three of the ravens were submitted to Prairie Diagnostic Services in Saskatoon to be evaluated for acetylcholinesterase (AChE) activity, and the results were as follows: - 2.07 µmol/min/g
- 1.50 µmol/min/g

Since the reported AChE activity in brain from healthy chickens is 15.99 +/- 3.18 µmol/min/g (1), these results indicated marked enzyme inhibition (less than 25% of normal activity), consistent with

organophosphorus (OP) or carbamate toxicosis. To further investigate the presumptive pesticide exposure, small pieces of soft tissues from the beef bones and liver from one raven were submitted to the Animal Health Laboratory for pesticide screening. Fenthion was identified in the liver sample by gas chromatography-mass spectrometry (GC-MS), while no toxins were found in the beef carcass. Since fish bones and scales were present in the gastric contents of one bird, it is possible that only the fish offal was poisoned, or perhaps scavengers consumed most of the meat on the beef bones. Regardless, it was speculated that the pile of animal remains was the most likely source of the pesticide because affected animals were in close proximity to it, and fenthion is highly toxic to birds.

Fenthion is an OP compound of intermediate oral acute toxicity in mammals (LD_{50} in rats = 255 mg/kg bw), but it is highly toxic to birds (LD_{50} = 2.5 mg/kg bw) and freshwater invertebrates (LC_{50} = 0.024 µg/L), creating potential risks for non-target species toxicosis. Organophosphorus compounds act by phosphorylation of a serine residue in the active site of AChE, causing "irreversible" enzyme inhibition. This leads to accumulation of acetylcholine at the synapse, overstimulating muscarinic and nicotinic cholinergic receptors and potentially resulting in a "cholinergic syndrome". Intoxicated animals and humans may present with increased salivation, lacrimation, urination, defecation, increased gastrointestinal motility, emesis and miosis (SLUDGEM); and in severe cases, convulsions, respiratory depression and death (2).

Fenthion-containing products were first registered in Canada in 1961 to control mosquitos, flies, birds and insects for companion animals and agricultural, industrial and residential buildings. Fenthion was not considered to pose an unacceptable risk to humans and the environment when used following the specifications, as concluded by the Canada Pest Management Regulatory Agency (PMRA) in a re-evaluation of fenthion (PACR2003-05) in 2003 (3). However, the manufacturer decided to discontinue these products in the USA and Canada. As a result, Health Canada provided a phase-out timeline for fenthion-containing products, allowing their use until December 31, 2006, as recommended by the PMRA in a subsequent re-evaluation of fenthion (RRD2004-10) in 2004 (3). Although any remnants of fenthion-containing products in Canada should have been disposed of thereafter, cases of toxicosis are still identified. *AHL*

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Leptospira spp. abortion in a Hanoverian mare

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A 13-year-old Hanoverian mare aborted December 1, 2021. The mare was up to date on vaccines with no recent history of illness, and no changes on farm beyond coming in at night starting November 15. The aborted fetus and placenta were submitted for postmortem examination at the AHL in Kemptville.

Postmortem findings for the fetus included icterus and marked subcutaneous edema, with small to moderate volumes of translucent amber-coloured fluid in the thorax and abdomen. Petechial hemorrhages were observed throughout the musculature of the body wall and across the epicardial surface, and the liver was markedly enlarged with small tan foci and fibrin scattered across the capsular surface (**Fig. 1**). The placenta was relatively unremarkable grossly, though mild edema was appreciated and the chorioallantoic surface appeared thickened and darker red in the pregnant vs the non-pregnant horn.

Histologically, there were clusters of mixed macrophages, lymphocytes and plasma cells in the epicardial connective tissue, extending slightly into the underlying myocardial interstitium. Scattered similar inflammatory cells, along with neutrophils, were present in sinusoids throughout the liver, along with frequent bile canalicular plugs and similarly pigmented hepatocytes. A definitive correlate for the tan foci observed grossly was not histologically apparent. A mixed population of inflammatory cells was also observed both within and surrounding capillaries in various organs including the stomach, cerebellum, intestines, and within lymph nodes and perinodal connective tissue.

The most notable histological changes were in the placenta where there was patchy marked edema of the chorioallantoic stroma and a mixed population of inflammatory cells scattered predominantly in perivascular positions throughout the chorioallantois. Scattered poorly preserved neutrophils were also observed admixed with cellular debris in the most severely-affected areas. Warthin-Starry silver stain demonstrated numerous argyrophilic spirochetes within the periphery of the edematous areas of the placental stroma (**Fig. 2A**). The same organisms were strongly immunoreactive for antibodies to *Leptospira* spp. (**Fig. 2B**). Kidney submitted for PCR was also positive for *Leptospira* spp.

The leptospiral placentitis resulting in both fetal hypoxia and systemic bacterial spread to the fetus was determined to be the underlying cause of the abortion. While icterus and interstitial nephritis are the most consistently reported pathological changes in aborted fetuses due to *Leptospira* spp., placental lesions similar to those described above have also been observed, and spirochetes have been consistently found in placental tissue (1). Mares that abort due to a leptospiral infection may present with no clinical signs. However, the bacteria can be shed for several weeks following abortion, and therefore could be of zoonotic concern on farm (2). *AHL*

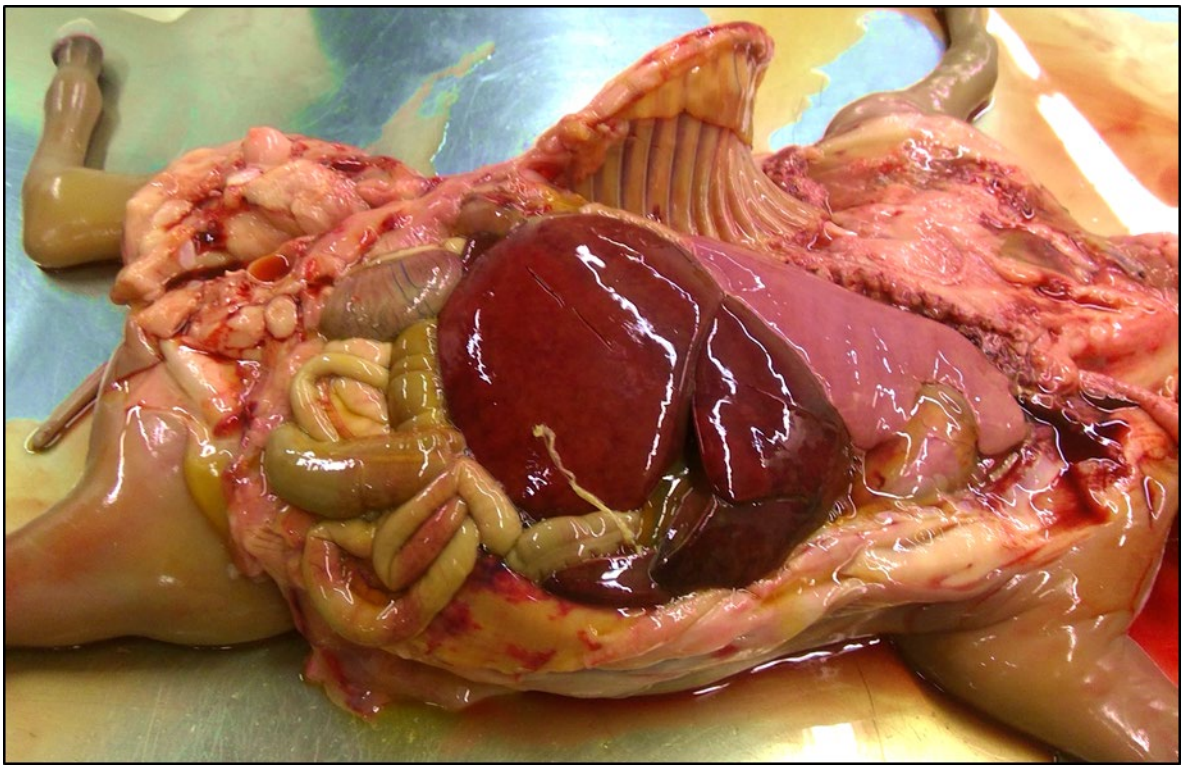

Figure 1. Aborted equine foal with icterus, subcutaneous edema, fibrinous abdominal effusion and thoracic effusion.

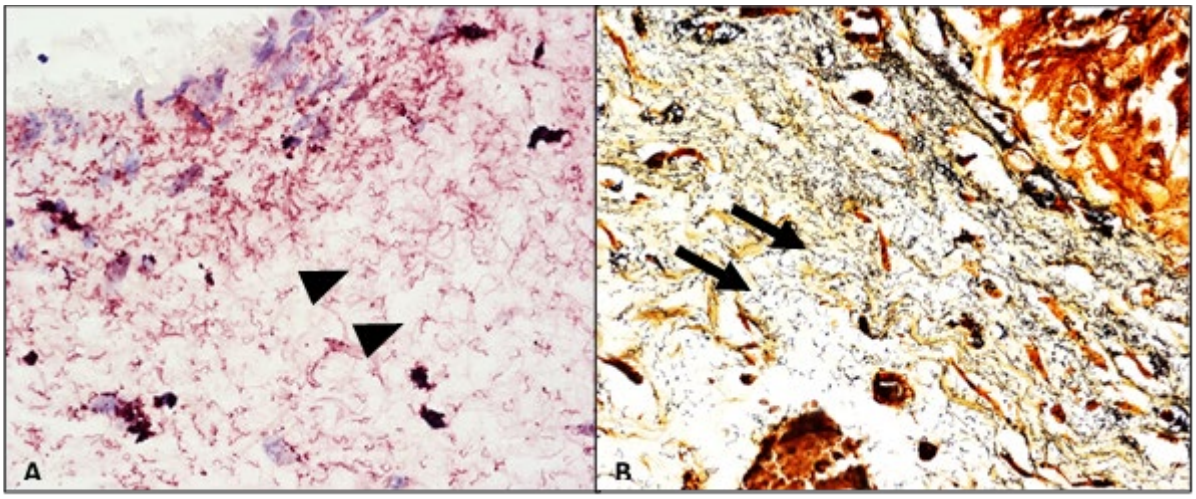

Figure 2. Leptospiral placentitis in a Hanoverian foal. Chorioallantois with immunohistochemistry for *Leptospira* (A) and Warthin-Starry silver stain (B). There is both positive red immunostaining (arrowheads) and silver staining (arrows) of innumerable individual leptospires in the edematous chorioallantois.

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Suspected copper storage hepatopathy in littermate Dalmatians

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Two deceased Dalmatian dogs were submitted to the Animal Health Laboratory approximately one month apart for postmortem examination.

The first dog presented to an emergency clinic for lethargy, vomiting and icterus following ovariohysterectomy surgery one week prior. *Leptospira* testing was negative. Clinical pathology testing identified marked elevation in aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and bilirubin. Euthanasia was elected.

The second dog presented to the same emergency clinic with a 24-hour history of vomiting and progressive lethargy. The dog was poorly responsive, laterally recumbent and had a short seizure. Clinical pathology testing revealed hypoglycemia, markedly increased ALT levels (beyond the analyzer limit), and prolonged prothrombin and partial thromboplastin times. *Leptospira* testing was negative. During assessment and treatment, the patient arrested, did not respond to cardiopulmonary resuscitation and subsequently died.

Postmortem examination identified marked icterus in both dogs, with subcutaneous petechiae in dog one, and extensive subcutaneous hemorrhage in areas of venipuncture for dog two. Both dogs had enlarged and discoloured brown/orange livers, with enhanced zonal patterns. Histologically, both livers had widespread acute midzonal to centrilobular hepatic necrosis with areas of panlobular necrosis. Special staining for copper identified widespread accumulation of fine granular pigment, consistent with copper, throughout all zones (**Fig. 1**). Quantitative copper levels were 1100 µg/g in both dogs, which is elevated from the reference range of 30-100 µg/g.

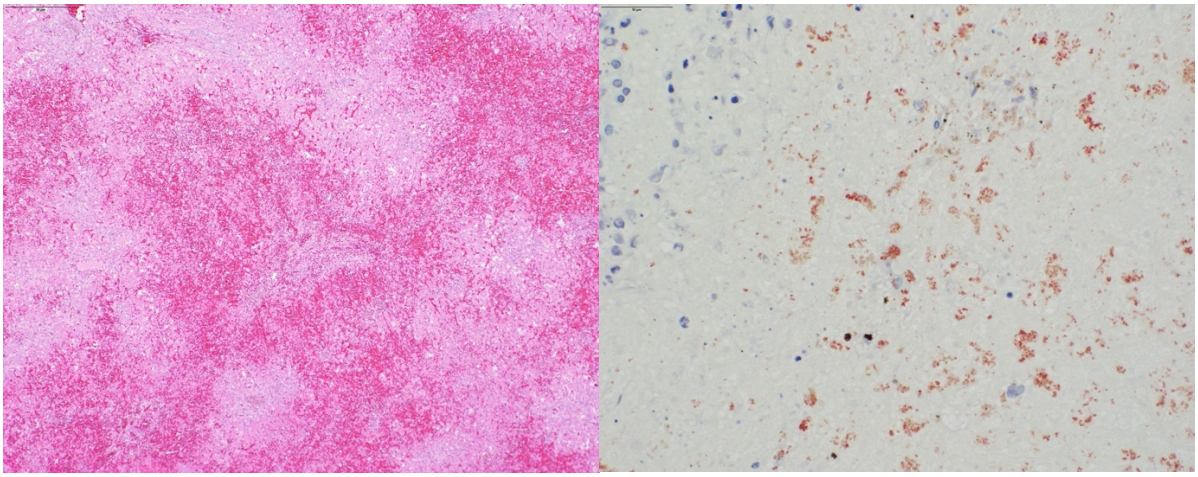

Figure 1. Widespread hepatocellular necrosis with hemorrhage (left, H&E stain); abnormal copper accumulation demonstrated by granular red pigment (right, rhodanine stain).

Following submission of the second dog, the emergency clinic communicated to pathologists at the Animal Health Laboratory that these animals were litter mates from separate households. Dog one had medication history (meloxicam) following routine spay, but no medication history was reported in the second dog. Toxicology testing (GC/MS) was requested for both dogs; results are pending. Given the breed, the similar clinical courses, and characteristic histologic lesions in combination with elevation of quantitative copper levels, the presumptive diagnosis for both dogs is copper storage hepatopathy.

Copper is an essential trace element that is required for various cellular functions. After absorption from the gastrointestinal tract, copper is transported to the liver where it is incorporated in enzymes or transported to extrahepatic tissues. Excess copper is typically secreted in the bile. In the event of abnormal accumulation or storage, excessive copper overwhelms lysosomal storage capacity and causes oxidative stress, free-radical formation and subsequent hepatocellular necrosis.

A specific genetic mutation that induces copper storage disease is well described in Bedlington terriers (COMMD1 deletion), and an underlying genetic cause has been proposed for other breeds including Labradors, Dalmatians, Dobermans and West Highland White terriers. Mutations in the ATP7B and ATP7A genes have been implicated in copper-mediated liver disease in Labrador retrievers, but the diagnostic utility in screening for these mutations is unknown, despite being commercially available. The genetic basis of disease in Dalmatians, while suspected, is not described.

Typically, definitive diagnosis of copper storage hepatopathy in dogs requires quantitative copper levels to exceed 2000 μg/g. In a retrospective study of 10 Dalmatians with presumptive copper storage disease, quantitative copper levels ranged from 754-8390 μg/g, with only 5/9 animals exceeding the 2000 μg/g threshold. In other types of copper-mediated associated liver injury such as chronic hepatitis, toxic levels of copper can vary, and an individual's threshold for hepatic injury is likely multifactorial and mediated by factors specific to that individual, i.e., genetic, physiologic, and environmental factors.

The presumptive diagnosis of familial copper storage disease in these two dogs is based on characteristic clinicopathological derangements, gross and histologic lesions, and demonstration of abnormal copper accumulation using histochemical staining. While the copper levels were not as high as expected for a definitive diagnosis, examining these cases in parallel strongly supports the diagnosis in these two littermates raised in separate households. *AHL*

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Hemoperitoneum as a manifestation of anaphylaxis in dogs

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Recent literature reports have described hemoperitoneum as a significant, potentially fatal outcome of anaphylaxis in dogs. In clinical cases where anaphylaxis is considered, evaluation for presence of

abdominal effusion and hemoperitoneum is warranted. Similarly, in dogs where hemoperitoneum is identified, inclusion of anaphylaxis as a differential diagnosis should be considered.

Since August 2021, hemoperitoneum has been identified in 3 dogs with possible anaphylaxis that were necropsied at the AHL. The dogs were young (1, 3, and 4 years of age), of various breeds and sex, and in good body condition. Acute collapse was reported in 2 dogs, both of which were outdoors at the time of the event. In the third dog, clinical signs included acute-onset ptyalism, pallor, diarrhea, and dyspnea. Each dog's condition rapidly deteriorated, culminating in death or euthanasia within 30 minutes to a few hours.

Postmortem lesions were similar in each dog. Abundant free fluid blood and few variably-sized blood clots in the abdominal cavity were estimated to represent 16% to 65% of each dog's total circulating blood volume. Prominent gall bladder edema was present in 2 of 3 dogs, and liver had a friable texture in 1 dog. There was no evidence in any of the dogs of hemorrhagic neoplastic or inflammatory abdominal mass lesions, of rupture of abdominal vasculature, or of hemorrhage at other anatomic sites. Two dogs had short, shallow capsular lacerations in spleen or liver that were unassociated with blood clots. It was suspected that these lesions resulted from resuscitation efforts or other physical manipulation, and the small size of the lesions was not considered significant to the development of severe hemoperitoneum. Toxicologic screening for anticoagulants was carried out on liver samples from 2 dogs, and no anticoagulants were detected.

Differential diagnoses for the causes of hemoperitoneum in dogs include blunt-force trauma, abdominal neoplasia (e.g., splenic hemangiosarcoma), acquired or hereditary coagulopathy, and aneurysm. Based on recent reports, anaphylaxis must also be considered as a differential diagnosis for hemoperitoneum in dogs. The clinical history provided for each dog in this series, and presence of gall bladder edema in 2 dogs, provide support for anaphylaxis in these cases. Anaphylaxis is difficult to confirm in dogs and other species, and diagnosis is often one of exclusion in the context of appropriate clinical signs and history.

The pathogenesis of hemoperitoneum associated with anaphylaxis is not well understood. The syndrome has been reported in 1 human case, in which hemorrhage was attributed to disseminated intravascular coagulation (DIC), and is not recognized in other species. In dogs, the liver and gastrointestinal tract are considered target organs for anaphylaxis. One effect of vasoactive mediators released during acute anaphylaxis is venous congestion, involving vena cava as well as other sites. As a result, gall bladder edema and hypoxic hepatic injury may develop, and these antemortem lesions may be detected ultrasonographically and by serum biochemical analysis, respectively. Increased vascular permeability and venous congestion in anaphylaxis may also act synergistically to promote hemoperitoneum. In addition, it is speculated that coagulopathy could be influenced by inflammatory mediators released by mast cells during anaphylaxis, although this mechanism is not fully elucidated. Coagulopathy resulting from insect stings has also been postulated as a cause of hemoperitoneum associated with anaphylaxis.
AHL

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