Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

Minimize staff time spent on out-of-province/country shipping and testing

Melanie Barham, Jim Fairles

AHL can facilitate hassle-free testing for out-of-province and out-of-country laboratory testing for all species! If one of your New Year's resolutions was for your team to simplify, let us help. The AHL not only provides high-quality testing in house, but is also happy to facilitate shipping to other accredited laboratories in the United States and Europe on behalf of clients. Sending testing to laboratories located long distances from your clinic is time-consuming with packaging, ensuring sample quality, and tracking, not to mention permit requirements for transporting potentially infectious samples cross-border. We are pleased to take the hassle out of this process for our clients.

With relationships with numerous accredited labs, we have expertise to send your samples quickly and obtain tracking updates and results in the same expeditious manner you are used to with the AHL. We maintain this service at as low cost as possible, with no markup on outside testing, and minimal shipping/repackaging fees.

Not sure if we can send to a particular lab? **Always remember that sending across borders can result in unexpected delays.** Please make sure that you allow adequate time before results are needed.

Call client services today 519 824-4120 x 54530, or ahlinfo@uoguelph.ca . *AHL*

Regulatory Amendments to O. Reg. 557 (Communicable Diseases – General)

Regulatory amendments to Ontario's *Health Protection and Promotion Act* have introduced new animal case reporting requirements for veterinarians across the province. **As of January 1, 2018, all veterinarians and directors of laboratories must report known and suspected cases of avian influenza, novel influenza and *Echinococcus multilocularis* infections in animals to their local public health unit**. Veterinary reporting requirements for **all mammal bites**, as well as all cases of **avian chlamydiosis** also continue to remain in place.

For further information about public health veterinary reporting requirements, please contact **Dr. Catherine Filejski**, Public Health Veterinarian, Ministry of Health and Long-Term Care (OMHLTC) at 416 212-0424 or catherine.filejski@ontario.ca .

The full text of the newly amended O. reg. 557/91 is also available online at https://www.ontario.ca/laws/regulation/900557?search=557 *AHL*

Jennifer Zoethout, Technical Supervisor, AHL Central Services

Following the completion of her BScH at the University of Guelph, Jen joined the Bacteriology section at the AHL as a Laboratory Technician. Within a few years she assumed the role of Team Leader in the Bacteriology section and continued in this position for over a decade.

As technical supervisor of AHL Central Services she takes great pride in ensuring that our clients receive the highest level of quality and service. *AHL*

AHL new tests and test panels developed in 2017

TEST NAME METHOD	CODE	SPECIES
Avian adenovirus - PCR	fadvrrt	Avian
Avian metapneumovirus type C (AMPV type C) - PCR	ampvpcr	Avian
Bacillus anthracis (anthrax) - real-time PCR	anthpcr	Bovine
Bacterial culture, aerobic and anaerobic, food animal	ancultf	Av, Bov, Cap, Ov, Porc, Other
Bacterial culture, honey bee, American foul brood (*P. larvae*)	cultbeo	Bees
Antimicrobial susceptibility, honey bee, *Paenibacillus larvae*	afbo	Bees
Bacterial culture, fecal, equine	cultnf1	Equine
Bacterial culture, fecal, *Campylobacter*, add-on test	campadd	Av, Bov, Cap, Eq, Other, Ov, Porc
Bovine abortion panel - PCR (BoHV-1/IBR, *Leptospira, Neospora*)	boabopc	Bovine
Bovine enteric panel - BCoV/RotaV A/B PCR; sucrose wet mount, bacterial culture	bentpnl	Bovine
Bovine respiratory virus panel - PCR (BoHV-1/IBR, BPIV-3, BRSV)	brvp3	Bovine
Bromethalin (desmethylbromethalin) - LC-MS/MS	dmb	Av, Bov, Can, Cap, Eq, Fel, Other, Ov, Porc
Haemoplasma, non-feline, non-canine - PCR *Mycoplasma haemolamae, M. ovis, M. suis, M. wenyonii*	hapcr2	Bovine, Other, Porcine
Leptospira spp. - PCR	leptpcr	Bov, Can, Cap, Eq, Fel, Other, Ov, Porc
Mycoplasma hyopneumoniae - gene sequencing typing	p146seq	Other, Porcine
Myxobolus cerebralis (whirling disease pathogen) - PCR	wdpcr	Fish
Rotavirus A&B/coronavirus, bovine/equine - PCR	rocopcr	Bovine, Equine
Rotavirus, group A, B, C – PCR	rotapcr	Canine, Other, Porcine
Scrapie resistance PrP genotyping, codons 136, 137, 154, 171, 176 - sequencing	prp5	Ovine

AHL Newsletter
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Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistants: **Helen Oliver, April Nejedly**

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.

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

Selected zoonotic pathogens and diseases from Ontario identified at the AHL, 2017

Beverly McEwen, Durda Slavic, Davor Ojkic, Hugh Cai, Kristiina Ruotsalo, Josepha DeLay, Margaret Stalker, Murray Hazlett, Andrew Brooks, Janet Shapiro, Maria Spinato, Marina Brash, Emily Martin

Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens in > 1,000 cases annually (**Tables 1 and 2**). The number and percentage of cases identified as positive for leptospirosis increased in 2017 in all species tested. These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as 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, 2017.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2017	2016	2015	2014	2013	2012
Ascarids (*T. canis, T. cati, T. leonina, Baylisascaris* sp.)	0	12	10	0	0	23	2	16	5	9	**77**	41	17	40	36	35
Blastomyces dermatitidis	0	0	0	0	0	0	0	13	1	2	**16**	6	21	22	17	10
Bordetella bronchiseptica	3	24	3	0	0	0	0	4	4	12	**50**	32	37	28	24	33
Borrelia burgdorferi (Lyme disease), serology	0	0	20	0	0	0	0	9	0	0	**29**	22	8	12	11	3
Brucella sp. *(non-abortus)*	0	0	0	1	0	0	0	0	0	0	**1**	3	0	1	0	0
Campylobacter coli/ jejuni/ fetus subsp. *fetus*	3	0	0	5	5	20	3	3	0	5	**44**	47	16	17	6	17
Chlamydia sp.	1	0	0	5	11	0	0	0	0	1	**18**	13	24	15	25	33
Clostridium difficile	1	8	4	0	0	0	0	0	0	1	**14**	5	10	11	11	19
Coxiella burnetii (Q fever)	9	0	0	39	54	0	0	0	0	0	**102**	37	44	55	28	36
Cryptococcus sp.	0	0	1	0	0	0	0	0	0	0	**1**	1	1	3	2	1
Cryptosporidium sp.	172	0	1	1	5	0	0	0	0	1	**180**	243	247	186	206	141
Eastern equine encephalitis virus	0	0	2	0	0	0	0	0	0	0	**2**	0	6	25	1	0
Giardia sp.	5	0	0	0	0	0	0	21	0	0	**26**	26	30	50	48	26
Listeria monocytogenes	19	0	0	4	8	0	0	0	0	0	**31**	24	12	23	15	18
Methicillin-resistant *Staphylococcus aureus* (MRSA)	2	0	9	0	0	0	0	0	0	0	**11**	12	28	17	8	24
Methicillin-resistant *S. pseudintermedius* (MRSP)	0	0	0	0	0	0	0	72	4	0	**76**	62	88	45	141	114
Rabies virus	0	0	0	0	0	0	0	0	0	0	**0**	2	0	0	0	0
Salmonella enterica	64	67	8	8	3	27	35	24	2	28	**266**	290	332	221	308	281
Streptococcus suis	10	102	2	2	1	1	1	0	0	1	**120**	181	167	105	126	144
Streptococcus equisimilis	4	28	14	1	0	0	0	8	1	3	**59**	57	48	4	34	45
Streptococcus zooepidemicus	0	2	121	2	0	0	0	2	2	4	**133**	154	138	93	112	4
Toxoplasma sp.	0	0	0	6	4	0	0	0	2	0	**12**	7	11	18	11	8
Verotoxigenic *E.coli* (VTEC)	5	0	0	0	1	0	0	0	0	0	**6**	12	8	7	18	
West Nile virus	0	0	15	0	0	0	0	0	0	43	**58**	18	19	6	44	36
Yersinia enterocolitica	2	6	0	0	0	0	5	0	0	0	**13**	4	2	6	4	2
Total	**300**	**249**	**210**	**74**	**92**	**71**	**46**	**172**	**21**	**110**	**1,345**	**1,299**	**1,314**	**1,010**	**1,236**	**1,030**

Table 2. Leptospira spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL, 2017.

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Other
L. autumnalis	27	7	18	86	2
L. bratislava	72	7	20	74	3
L .canicola	39	7	8	50	0
L. grippotyphosa	16	6	4	71	1
L. hardjo	50	7	3	31	2
L. icterohaemorrhagiae	55	6	15	82	1
L. pomona	64	7	11	71	5
IHC, or urine PCR-positive	1	1	0	10	0
Positive/tested cases	**91/178**	**8/27**	**26/35**	**153/230**	**5**
% positive, 2017/2016	51/27%	30/9%	74/52%	67/38%	

ONTARIO ANIMAL HEALTH NETWORK

HIGHLIGHTS 2017

OAHN FUNDED PROJECTS

Addressed a surveillance gap for the species

$50,000 per species group was awarded

Concluding now- final reports to be posted soon!

Check social media & our website for features on each research project.

DATA & INTERPRETATION

Expertise to assist with data interpretation present on network calls

Annual review of AMR data

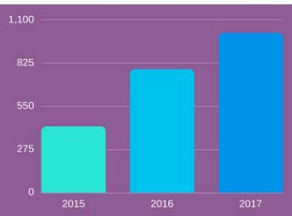
New data sources were obtained based on industry needs

Log in to the vet side of OAHN.ca to view data discussed on network calls.

GROWING ONTARIO AUDIENCE

>1300

Podcast listens per quarter in 2017



Facebook followers

7184

Average hits on our website in 2017

>65% of our audience is from Canada for **ALL** platforms, mostly from Ontario.

NEW TOOLS

OAHN blog started in 2017 with tips on website, how OAHN interacts with other agencies etc

Weekly surveillance scan report

Resource pages on specific diseases (tick-borne, PED etc)

We develop communication tools to meet evolving needs of the networks.

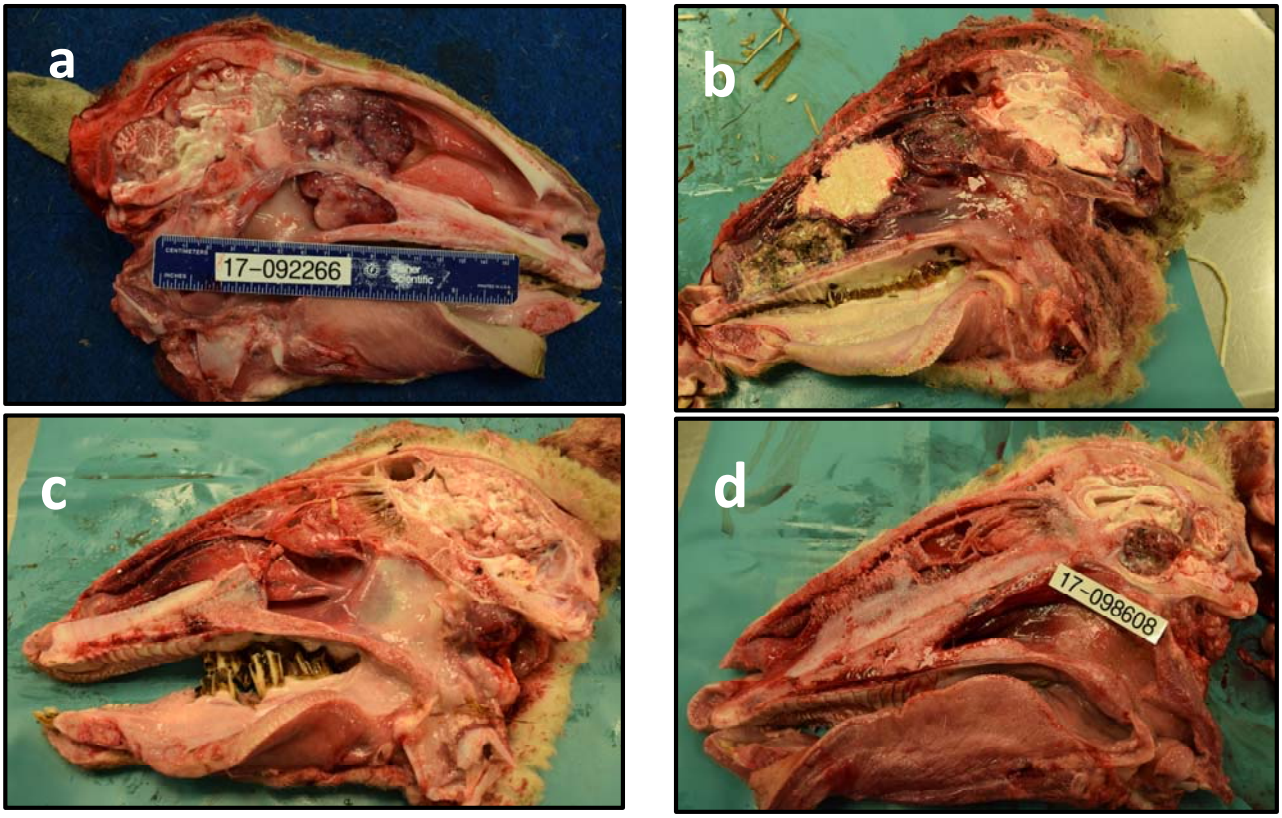
WWW.OAHN.CA

AHL Lab Reports

RUMINANTS

What is your diagnosis? *Maria Spinato*

(Hint – the answer is in the head! Answers on page 6.)



The protocol for on-farm postmortems performed under the **Small Ruminant Adult Mortality Project** includes submission of the intact head, in addition to a list of fresh and formalin-fixed tissues. Upon receipt at the AHL, pathologists split the head, examine and photograph it, and collect the brain for ancillary testing. If lesions are identified in the oral or nasal cavities, these are described and samples are obtained for analysis. Above are 4 recent examples. Answers on page 6. *AHL*

Salmonella Dublin ELISA now verified and available

Jim Fairles, Davor Ojkic

With the use of both confirmed positive and negative sera received from another AAVLD-accredited lab (Animal Health Diagnostic Centre, Cornell University), and positive and negative sera results confirmed in an interlaboratory comparison with the same lab, the AHL has now verified the Prionics *Salmonella* Dublin antibody ELISA in-house and is accepting samples.

***Salmonella* Dublin antibody ELISA**
Fee - **$9.50**
Sample - 1mL serum
Code - **salmdel**
Turnaround time- 1-5 business days

Coxiella burnetii in bovine abortion submissions

Andrew Brooks, Murray Hazlett, Beverly McEwen, Janet Shapiro, Josepha DeLay, Maria Spinato, Margaret Stalker, Andrew Vince, Emily Brouwer

From 2011 to 2017, *Coxiella burnetii* was detected in 15 submissions to the AHL that involved bovine reproductive losses. The majority of cases (10 of 15) involved abortions in dairy cattle. Two cases were stillbirths and one case involved a weak calf that died shortly after delivery.

C. burnetii is an obligate intracellular bacterial pathogen that is often associated with abortion in sheep and goats, but there are few reports of this organism causing abortion in cattle. Determining the significance of *C. burnetii* in cases of bovine abortion can be challenging. Serologic evidence suggests that the organism may be widespread in Ontario cattle herds, and asymptomatically infected cows can shed the organism in the placenta during normal parturition.

Pathologists interpreted the presence of *C. burnetii* as either suspicious or significant in 10 cases in this series. In the remaining cases, the organism was considered to be an incidental finding or to have questionable significance.

Placentitis was the lesion most frequently associated with *C. burnetii*, characterized by various degrees of necrosis, fibrin exudation, and neutrophil or mononuclear leukocyte infiltration (**Fig. 1**). In many submissions, other pathogens were also present that may have contributed to the placentitis, including *Ureaplasma*, *Bacillus licheniformis*, *Mycoplasma* spp., *Streptococcus pluranimalium*, *E. coli*, and *Klebsiella pneumoniae*.

C. burnetii was detected by quantitative PCR (qPCR) performed on the placenta in 13 submissions. In 2 submissions that lacked placenta, the organism was detected by qPCR performed on fetal lung or stomach content. In some cases, the organism was visible in placental trophoblasts in routine H&E-stained sections and was detected in modified acid-fast stains of placental smears.

The range of *C. burnetii* concentrations in the placenta, fetal lung, or stomach content, as measured by qPCR, varied considerably from 10^0 to 10^7 copies/µL. **The highest concentrations of *C. burnetii* were generally found in the placenta, and concentrations tended to be greater in those cases where the pathologist concluded that the organism was significant or suspicious.** However, further research is required to determine whether there are correlations between *C. burnetii* concentration, placental pathology, and abortifacient role. *C. burnetii* test results in cases of bovine abortion must be interpreted in light of the pathology findings and presence or absence of other abortifacient pathogens.

As the cause of Q fever, *C. burnetii* is an important zoonotic pathogen. The presence of *C. burnetii* in fetal and placental tissues from bovine abortions highlights the potential zoonotic risk associated with handling these materials. The AHL will continue to monitor for *C. burnetii* in bovine abortion submissions. Please contact the AHL if you have any questions about sample or test selection for abortion cases - helpful guidelines are published in the AHL User's Guide and sample collection templates are available from the Ontario Animal Health Network. *AHL*

References

Bildfell RJ, et al. *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. J Vet Diagn Invest 2000;12:419-425.

Agerholm JS. *Coxiella burnetii* associated reproductive disorders in domestic animals – a critical review. Acta Vet Scand 2013;55:13.

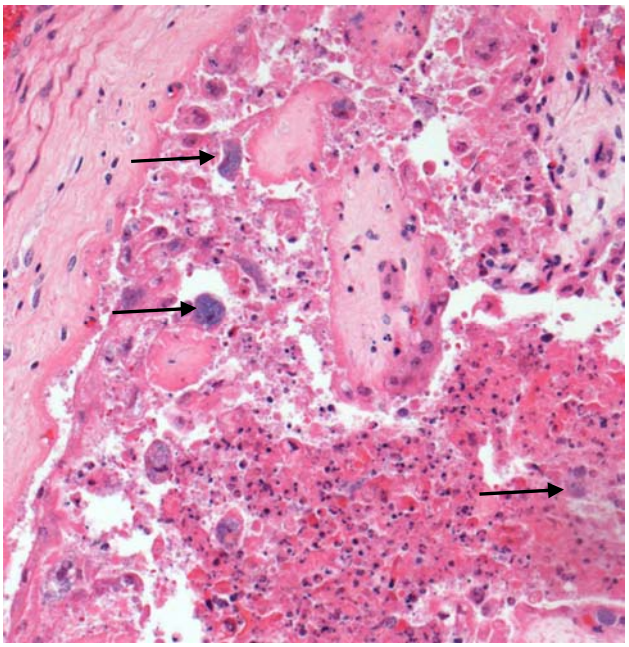


Figure 1. Bovine placentitis, with *Coxiella* organisms (arrows).

Answers to *What is your diagnosis*?

a) 2-year-old ewe: **nasal adenocarcinoma**, caused by enzootic nasal tumor virus (ENTV).
b) 6-year-old ewe: **nasal abscess.**
c) 6-year-old ewe: **severe dental disease causing emaciation.**
d) 6-year-old ewe: **pituitary adenoma.**

SWINE

Leptospiral abortion in swine

Josepha DeLay, Murray Hazlett, Maria Spinato, Davor Ojkic

Three recent swine abortion cases submitted to the AHL were suspicious for *Leptospira* infection as the cause of abortion. **The clinical histories included sporadic abortions with stillbirth and mummification.** In 2 of the cases, no other significant pathogens were identified to explain abortion, and overall test results were considered supportive of leptospirosis as the cause of abortion.

Leptospira titers in fetal thoracic fluid were mildly (2 cases) to markedly (1 case) elevated, with titers of 20-320. *Leptospira* nucleic acid was detected in low level by PCR in placenta from 1 case. No gross or histologic lesions suggestive of leptospirosis were evident in fetal kidney, liver, placenta, or other tissues from any of the cases. Maternal serology was pursued in 2 cases; in 1 of these, significantly elevated titers to *L. grippotyphosa, L. canicola, L. hardjo,* and *L. icterohaemorrhagiae* were identified among aborting sows compared to herdmates that farrowed normally. It is speculated that inconsistent vaccination against *Leptospira* may have contributed to infection and abortion.

Diagnosis of leptospiral abortions can be challenging and relies on multiple test methods. Exclusion of other causes of abortion is necessary. Even with a full range of testing, a diagnosis of leptospirosis often remains presumptive. Tests for *Leptospira* are an important part of the abortion workup and include:

- **fetal serology** (*Leptospira* microscopic agglutination test (MAT) using fetal thoracic fluid)
- ***Leptospira* PCR** (fetal kidney and placenta)
- **histopathology** and *Leptospira* **immunohistochemistry** (IHC)

Maternal serology (MAT) is a valuable second-tier test if initial results are suspicious for leptospirosis. Serum samples from 5 affected (aborting) and 5 unaffected cohorts (at the same stage post-*Leptospira* vaccination as the aborting sows) may be submitted for *Leptospira* MAT. Maternal vaccination complicates interpretation of serology results. Titers >1,600 are likely not associated with vaccination and represent recent infection, based on our experience at the AHL. A significantly higher titer for 1 or more *Leptospira* serovars among the aborting sows will add further support as the cause of abortion, and may indicate the serovar most likely responsible. **Cross-reactivity among serovars is common**, as is likely in the case described above. Importantly, only the serologic test will provide information on specific serovars potentially contributing to infection, given that current PCR and IHC tests can only identify *Leptospira* at the genus level.

For all infectious causes of abortion in swine, examination and testing of multiple fetuses from multiple litters will increase the likelihood of identifying an infectious agent, if present. Submission of 3 fetuses from 3 separate litters for postmortem is recommended. Samples are pooled by litter for efficiency and economy. *AHL*

Update on AHL *Mycoplasma hyopneumoniae* molecular typing

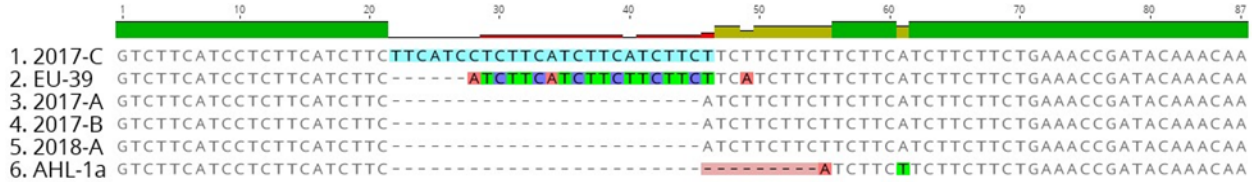
Hugh Y. Cai, Qiumei You

With support from the OMAFRA-AHL Disease Surveillance Program, we implemented a *Mycoplasma hyopneumoniae* molecular typing assay using *p146* gene-sequence analysis last year (See September 2017, AHL Newsletter). The following are some case examples: *M. hyopneumoniae* positive samples 2017-A, -B, -C and 2018-A were from different premises. The client wanted to know if these strains were related.

The results: ***p146* gene sequence analysis indicated that samples 2017-A, -B and 2018-A contained identical *M. hyopneumoniae* DNA with 100% similarity.** All 3 strains were different from strain 2017-C with 88% similarity and had 24 base pairs of "TC repeat" deletion. Compared to the AHL historical isolates, the 3 identical strains were most closely related to group AHL1a with 94% similarity. Among the 83 European strain types, the closest stain was strain type 39 (EU-39), which had 90% similarity to 2017-A, -B and 2018-A, and 96% similarity to 2017-C (**Fig. 1**).

Based on the above preliminary results, the *p146* sequence analysis appears to be useful for *M. hyopneumoniae* field strain typing. **The AHL continues to offer this assay for half-price ($40/sample) until May 1, 2018**. *AHL*

Figure 1. Sequence alignment of partial *p146* gene of recent and close related historical Ontario and European *M. hyopneumoniae* strains.



AVIAN/FUR/EXOTIC SPECIES

Where can non-poultry veterinarians in Ontario access small poultry flock resources?

Al Dam, Melanie Barham, Marina Brash

Interest in small poultry flocks continues to increase for many reasons, including the local food movement, with **over 16,000 non-quota/non-commercial poultry flocks registered with the Chicken Farmers of Ontario in 2016**, representing a 7% increase over 2015. Spring is a common time to add to flocks with chicks, goslings, or ready-to-lay birds. When small flock producers encounter problems, their first instinct is to search for advice from many sources including contacting their local veterinarian who may not be well-versed in small flock poultry concerns.

The need for small poultry flock veterinary care will continue to sharply increase for, **as of December 1, 2018, antimicrobials will no longer be sold at livestock medicine outlets in Canada.** This means that all farm animal producers, including small flock owners, will need to have a veterinarian-client-patient relationship (**VCPR**), as a prescription will be required to obtain antimicrobials.

So where can non-poultry veterinarians in Ontario access resources to assist with small flock testing and management information?

You don't have to "wing it", help is available!

Ontario Animal Health Network (OAHN) small flock veterinary listserv: (FREE) listserv for veterinarians interested or working on small flock poultry. Veterinarians can email the group to access help from experienced poultry practitioners, and over 40 other practitioners interested in small flocks. Email oahn@uoguelph.ca to be added to the listserv.

OAHN small flock poultry online veterinary course: (FREE) Veterinarians may access this free course and associated resources by logging in to www.oahn.ca (obtaining an account is free and easy). Then click this link to access the recorded lectures from this one day course. http://oahn.ca/resources/poultry/small-poultry-flock-workshop-for-veterinarians-presentations-live-video-and-resources/

VIN: Veterinary Information Network has case help for small flock poultry veterinarians, including a course, calculator for adding medications to water, euthanasia and more. Search small flock poultry on VIN's page for info.

OMAFRA: OMAFRA resources are located here: http://www.omafra.gov.on.ca/english/livestock/poultry/smallflock.html

FREE Poultry Biosecurity Kit - Keeping your Birds Healthy, Biosecurity Basics for Backyard Flocks, which can be accessed at: http://www.omafra.gov.on.ca/english/livestock/poultry/facts/hbresourcekit.htm

Animal Health Laboratory: AHL can answer diagnostic questions, and also has a large number of LabNotes on best practices for submissions. https://www.uoguelph.ca/ahl/

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Infectious bronchitis virus infection in Ontario 2014-2017

Davor Ojkic, Emily Martin, Marina Brash, Margaret Stalker, Leonardo Susta

Infectious bronchitis virus (IBV) infection of chickens in Ontario and Canada was for many years limited to sporadic outbreaks. Starting in early 2012 and in 2013, increased numbers of IBV-associated cases have been reported. These cases involved respiratory disease, increased mortality, urate nephrosis, and egg-production drops, and were associated with incursion of IBV strain 4/91. In **2016 and 2017, IBV re-emerged as the most important viral pathogen of chickens in Ontario,** generating a marked increase in submissions related to IBV infection (**Fig. 1**). **IBV infection caused severe disease and high losses affecting all commodity groups.**

Based on sequence comparisons of the hypervariable region of the S gene from 505 IBVs, field strains detected in Canada from 2014-2017 could be divided into 5 major groups: 1) vaccine-like, classic viruses, such as Connecticut and Massachusetts; 2)" indigenous" Canadian variants not described elsewhere, such as strain Qu_mv; 3) variant viruses related to strains described in the US, such as DMV/1639/11, California 1734/04, CU_82792/GA98 and Pennsylvania Wolg/98; 4) exotic, non-Canadian, non-US viruses, such as strain 4/91 (793b); and 5) "untypable" IBVs not showing significant similarity to previously described IBV strains (**Table 1**).

Massachusetts-type vaccines are the primary IBV vaccine type used in Canada, occasionally used in combination with Connecticut -derived strains. These vaccines can provide cross-protection against various IBV variants, presumably due to cross-reactivity involving cytotoxic T lymphocytes. However, recent incursions of 4/91, California 1734/04, and DMV/1639/11-like viruses were associated with various and often severe disease processes in all chicken commodity groups. It appears that challenge with these "new" viruses cannot be controlled by vaccines currently available in Canada, and alternative vaccination protocols may need to be considered. *AHL*

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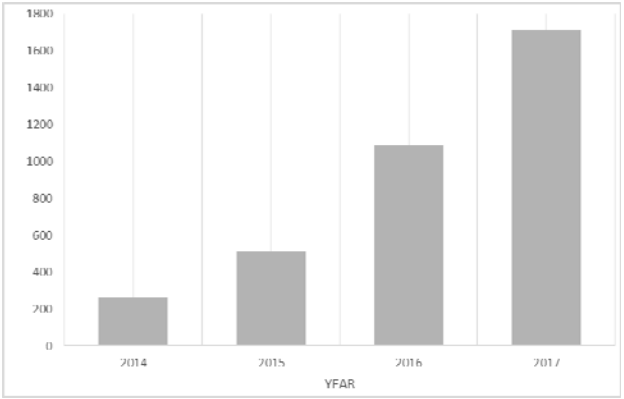

Figure 1. Number of samples submitted for testing for IBV by PCR from 2014-2017.

Table 1. Summary of incidence of IBV strains genotyped from 2014-2017.

	Strain	2014	2015	2016	2017
1) Vaccine-like	**Mass**	42.9%	43.7%	31.5%	**28.3%**
	Conn	7.1%	25.4%	4.6%	**6.5%**
2) Indigenous Canadian	**Qu_mv**	0.0%	0.0%	3.8%	**1.4%**
3) US-variant	**CA 1737 04**	7.1%	14.1%	20.8%	**9.4%**
	CU82792	0.0%	4.2%	0.8%	**1.4%**
	DMV	0.0%	1.41	23.8%	**46.0%**
	GA 2012	0.0%	4.2%	1.5%	**0.4%**
	PA_Wolg	0.0%	0.0%	2.3%	**0.0%**
4) Exotic	**4_91**	28.6%	5.6%	6.2%	**2.9%**
5) Untypable	**Not typed**	14.3%	1.4%	4.6%	**3.6%**

HORSES

Alcohol and Gaming Commission of Ontario (AGCO) Equine Incidents in Ontario Racing: 2003 - 2017 postmortem summary

The Alcohol and Gaming Commission of Ontario (AGCO; formerly the Ontario Racing Commission, ORC) continues in its **proactive approach to advance racehorse welfare and safety of human and animal participants**. In 2003, Ontario became one of the first North American racing jurisdictions to require mandatory reporting of racehorse deaths, in order to monitor, research and improve knowledge of why these events occur. Postmortem (PM) exams conducted at the Animal Health Laboratory through the AGCO Equine Incidents program continue to provide comprehensive data regarding the causes of morbidity and mortality in racehorses in this province. To date, PM has been carried out on 1075 horses through the Equine Incidents program (**Table 1**). Annual variation in the number of PM cases reflects discretionary requirement for PM on the part of the Registrar of AGCO.

A summary of significant PM findings is provided in **Table 2**. A comprehensive review of AGCO PM cases was conducted in 2015 as part of a separate retrospective study and as a result, some cases have been reclassified from results presented in previous editions of the AHL Newsletter. Results of the study were published in the July 2017 edition of the Journal of Veterinary Diagnostic Investigation.

Since 2015, **computed tomography (CT) of fractured and contralateral limbs** has been carried out on select Equine Incidents postmortem cases through collaboration with the Diagnostic Imaging section of the Ontario Veterinary College Health Sciences Center. The goal of this in-depth examination is to identify pre-existent lesions, primarily in bone, that contribute to catastrophic fractures. The procedure was continued in 2017, with CT imaging of 31 of 34 (91%) limb fracture cases submitted for PM exam. **Pre-existent lesions in bone were identified by CT and considered predisposing to fracture in 15 of 31 (48%) cases.**

Exercise-associated sudden death is of special concern among those cases reported through the Equine Incidents program (**Table 3**). In 2017, the cause of death was investigated in 9 horses that died while exercising. Of these, significant pulmonary hemorrhage was evident in 6 horses, and no cause of death was identified in 3 horses. Among all sudden death cases from 2003-2017, significant pulmonary hemorrhage was identified in 84 of 172 (49%) of horses. The cause of death in such cases is often attributed to exercise-induced pulmonary hemorrhage (EIPH), although the pathogenesis of pulmonary hemorrhage in these horses is not well understood. Severe acute hemorrhage involving pericardium or body cavities was identified in 30 of 163 (18%) sudden death cases in previous years, but this finding was not present in any of the sudden death horses examined in 2017. In a significant proportion of exercise-associated sudden death cases from 2003-2017, no significant lesions were identified and the cause of death remained undetermined (40/172, 23%). It has been speculated that **exercise-associated cardiac arrhythmia**, leading to acute heart failure and pulmonary hypertension, may be the underlying cause of death among many of these horses, and may also contribute to pulmonary hemorrhage in these animals.

Summaries of postmortem submissions to the Animal Health Laboratory under this program and diagnoses by body system for these cases are provided in the tables on page 11. *AHL*

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AHL accepts EIA electronic submissions via EquusLINK *Jim Fairles*

You can learn more about EquusLINK at: https://www.globalvetlink.com/products/equuslink/

Advantages of this electronic submission process include:

- Faster form completion, including immediate updating of information for correction of omissions.
- Streamlined submission to the lab, including embedded color photographs of horses rather than hand drawings.
- Electronic reporting of results, and streamlined transmission of test results to CFIA.
- All results stored for immediate retrieval and immediate use (no more mailing results).

Traditional EIA submission forms will of course still be accepted.

Note from the CFIA accredited veterinarian's manual: http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/accredited-veterinarian-s-manual/chapter-3/eng/1345233051622/1345233162747?chap=2

> "42. The shipment of samples must never be entrusted to the animal owner or exporter. The accredited veterinarian must be able to maintain a chain of custody for samples shipped to the laboratories."

After-hours drop-offs at AHL-Guelph require authorized signatures to maintain chain of custody. *AHL*

AGCO Death Registry—continued.

Table 1. Breed distribution of AGCO Equine Incidents submissions to the AHL, 2003-2017

Breed / year	Standard-bred	Thorough-bred	Quarter Horse	Total
2003	63	59	0	**122**
2004	81	60	0	**141**
2005	59	51	0	**110**
2006	58	46	2	**106**
2007	66	53	3	**122**
2008	27	24	0	**51**
2009	28	16	1	**45**
2010	22	8	2	**32**
2011	24	18	4	**46**
2012	20	14	0	**34**
2013	19	26	2	**47**
2014	21	22	8	**51**
2015	29	24	3	**56**
2016	15	32	3	**50**
2017	26	34	2	**62**
Total	**558**	**487**	**30**	**1075**

Table 2. Significant postmortem lesions identified in AGCO Equine Incidents submissions by body system, 2003-2017.

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

Table 3. Significant postmortem lesions recorded in exercise-associated sudden death cases, AGCO Equine Incidents, 2003-2017.

Body system affected and significant lesions / cause of death	Cases	Total
Respiratory		
EIPH	68	
Pulmonary hemorrhage (not classified by pathologist as EIPH)	16	
Miscellaneous	3	**87**
Cardiovascular		
Body cavity or pericardial hemorrhage	17	
Aortic rupture and cardiac tamponade	13	
Miscellaneous cardiac lesions	5	**35**
Cause of death undetermined		**40**
Skull fracture (potentially secondary to collapse)		**5**
Sepsis / disseminated intravascular coagulation		**5**
Total		**172**

A. Equine metabolic syndrome testing

Kris Ruotsalo, Felipe Reggeti

Equine metabolic syndrome (EMS) has been described as a condition affecting young to middle-aged horses, commonly associated with **insulin dysregulation** (ID), and often with altered circulating adipokine concentrations, dyslipidemia, predisposition to laminitis and possible regional or generalized adiposity.[1]

Insulin dysregulation encompasses both tissue insulin resistance, and persistent or intermittent hyperinsulinemia without concurrent tissue insulin resistance. In the clinical setting, **insulin status may be easily assessed by "resting" or "dynamic" testing** (**Fig. 1**). Increased baseline serum insulin concentration in horses without access to grain for 4 hours supports hyperinsulinemia. The test has low sensitivity but high specificity; however, **it is important to keep in mind that conditions other than EMS can cause hyperinsulinemia, including pituitary pars intermedia dysfunction (PPID), pregnancy, stress, illness, and high energy forage**. A result within reference intervals in a horse with strong clinical signs of EMS is considered equivocal. In these cases, dynamic testing is recommended due to higher sensitivity, including the oral sugar test (for details, please see: http://sites.tufts.edu/equineendogroup).

Serum glucose concentration by itself is insensitive and influenced by many factors, thus concomitant testing for serum glucose and insulin concentrations is recommended.

Ancillary tests that may be considering in the assessment of horses with EMS include leptin, a hormone produced by adipocytes. The hormone is not specific for EMS, as it may be elevated with an increased body condition score but it may be helpful to assess internal adiposity and/or to monitor response to management. In addition, a marked increase in serum leptin values has been shown to correlate with the future development of laminitis.

As horses with EMS age, concurrent PPID may develop; however, to our knowledge, there are no currently published studies establishing a causal relationship between the 2 conditions. Because PPID may also be associated with insulin dysregulation, testing endogenous ACTH, insulin and glucose concentrations may be indicated if clinical signs are supportive. *AHL*

Reference

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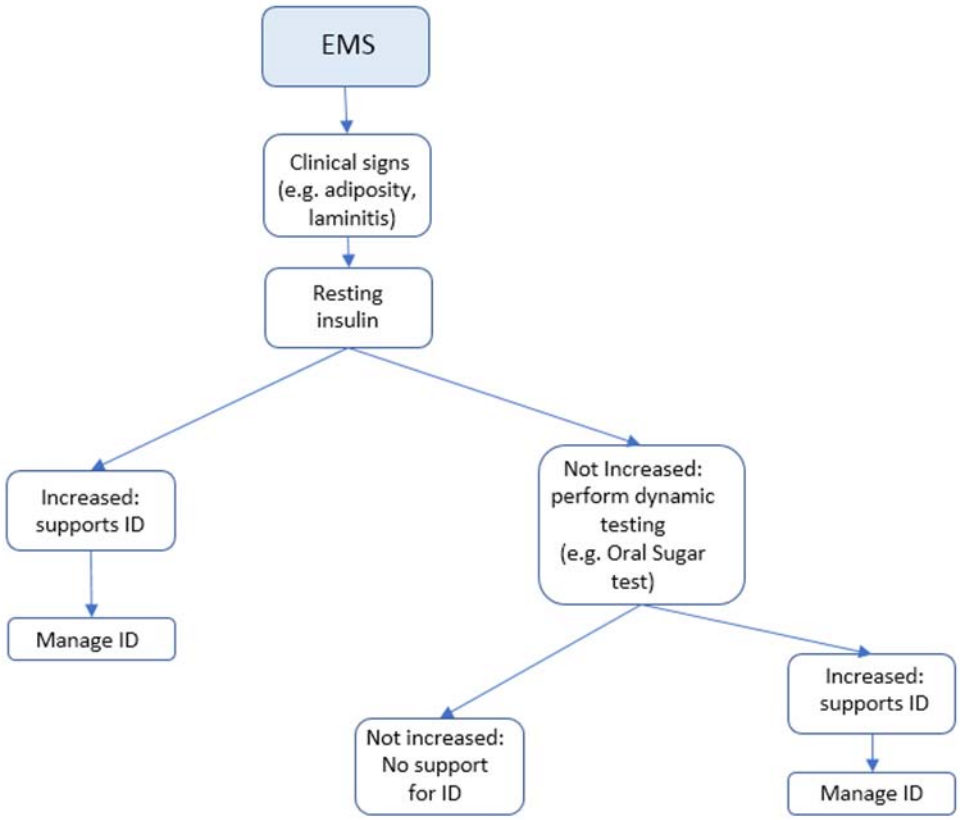

Figure 1. Assessment of insulin dysregulation (ID) - adapted from the Equine Endocrinology Group recommendations.

B. Pituitary pars intermedia dysfunction (PPID) testing

Kris Ruotsalo, Felipe Reggeti

Pituitary pars intermedia dysfunction (PPID; "Cushing's disease") is an endocrine condition commonly identified in aging horses and ponies. It is considered to result from loss of dopaminergic inhibition of the pituitary gland causing excessive release of ACTH into plasma and subsequent hypercortisolemia. The diagnosis may be based on clinical signs in "full-blown" cases, but it is often more difficult in animals with subtle signs or inconclusive laboratory data.

Multiple endocrinology tests have been proposed to support a diagnosis of PPID. The 2017 Equine Endocrinology Group (http://sites.tufts.edu/equineendogroup) recommendations **suggested the use of endogenous ACTH for moderate and advanced cases of PPID, and the TRH-stimulation test for equivocal or early PPID** (**Fig. 2**). Protirelin (a synthetic analog of TRH) may be available from veterinary compounding pharmacies, however it is advisable to contact the pharmacy directly to confirm, and establish pricing.

The AHL offers a **chemiluminescent ACTH test**, individually and within endocrine profiles. There are no fasting requirements related to sampling, however specific sample handling requirements must be adhered to. In addition to PPID, endogenous ACTH may be increased with other illness, excitement and stress.

A "**seasonal increase**" in ACTH has been documented during the fall months, with median concentrations ~2 times the upper reference limit (although some healthy horses showed significantly higher values). This seasonal increase has an impact in the interpretation of laboratory results. Reference intervals at the AHL (2-10 pmol/L) were developed outside the seasonal rise; thus, as an example, an ACTH result of 18 pmol/L could be unremarkable in the fall but would be interpreted as increased for the remainder of the year. The seasonal rise in ACTH may also be exaggerated in early PPID, increasing the sensitivity of the test, however seasonally adjusted reference intervals would be required for accurate interpretation.

Further, **some of the clinical and laboratory findings of PPID overlap with other endocrine conditions, such as equine metabolic syndrome, occasionally making diagnostic interpretation challenging.** *AHL*

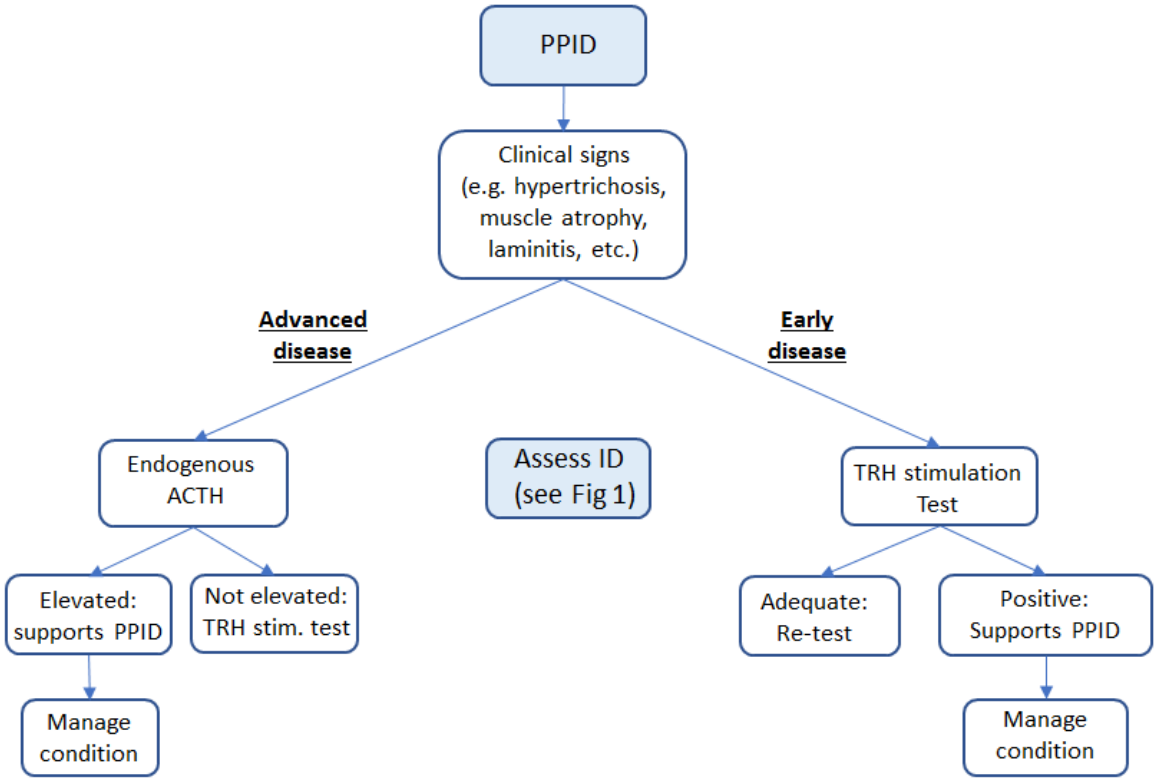

Figure 2. Assessment of PPID - adapted from the Equine Endocrinology Group recommendations.

COMPANION ANIMALS

Post-surgical death and acute thymic hemorrhage in a young dog

Jan Shapiro, Kris Ruotsalo

A 6-mo-old male Golden Doodle dog was submitted to the Animal Health Laboratory in Kemptville for postmortem. Three days prior to death, the dog had undergone surgery for routine castration, had recovered from anaesthesia uneventfully, and was discharged from the clinic the same day. Pre-anesthetic hematology had shown no abnormalities. The day after surgery, the dog was re-presented to the clinic, as the owner was concerned about continuous dripping of blood from the castration incision. On clinical examination, the attending veterinarian noted excessive bruising of the scrotum, and when questioned, the owner mentioned that they felt that the dog had experienced excessive bleeding when it was teething. The following day, bleeding from the surgery site seemed to have stopped, but the dog was weak, lethargic, and had an increased respiratory effort. Oral mucosa was pale, and scrotal bruising was worse. A CBC showed low values for red blood cell counts, and low hematocrit, hemoglobin, MCHC, and total protein, with a platelet count within the normal range. Anemia due to blood loss was diagnosed, and the decision was made to send the dog to a referral clinic, but the dog died en route.

At postmortem, there was extremely marked generalized pallor. The thoracic cavity held ~ 175 mL of unclotted blood. In the left cranial thorax, there was an **8 x 5 x 2 cm solid hematoma dissecting throughout the thymus** (**Fig. 1**), leaving various sized islands of compressed pale lobules. The adjacent mediastinum and the pericardial sac were markedly expanded with clotted blood, and ~ 2 mL of unclotted blood was in the pericardial sac. Dissecting hemorrhage extended cranially in the soft tissue encircling the distal 1/3 of the trachea. Dissecting hemorrhage also encircled the intra-thoracic aorta, vena cava, and esophagus. No abdominal hemorrhage was observed. There was marked swelling of the scrotum with a large solid hematoma, and the scrotal skin, including the incision which had focally dehisced, was covered with dried blood. Hemorrhage extended cranially for several cm into the subcutis and fascia of external abdominal oblique muscle and into the soft tissue around the penis, but did not extend into the abdominal or pelvic cavities. A specific vascular site of origin of the scrotal hemorrhage could not be found.

The postmortem diagnosis was severe anemia associated with post-castration scrotal hemorrhage, and thymic hemorrhage, with severe secondary hemothorax, hemopericardium and hemomediastinum. Histopathology of the thymus showed hemorrhage.

Severe thymic hemorrhage or thymic hematoma is a well-recognized condition of young dogs that is often fatal because of hypovolemic shock secondary to massive mediastinal hemorrhage. Reported causes of thymic hematoma include severe trauma directly to the thorax, or to the thymus by over-extension of the neck or excessive pulling on the dog's collar, dissecting aortic aneurysms, bleeding thymic neoplasms, and various coagulation disorders, including vitamin K- responsive coagulopathy and anticoagulant rodenticide toxicosis. Spontaneous idiopathic thymic hemorrhage of young dogs 3-9 mo of age is a rare disease, diagnosed by exclusion of other causes, with the underlying predisposing factor being fragility of the thymus associated with age-associated atrophy.

The reason for the thymic hemorrhage could not be confirmed in this dog. A pre-existing coagulation disorder was suspected, based on the owner's comment about previous excessive teething hemorrhage, but could not be proven without corroborating clinical pathology and coagulation profile data. Toxicology testing was not performed.
AHL

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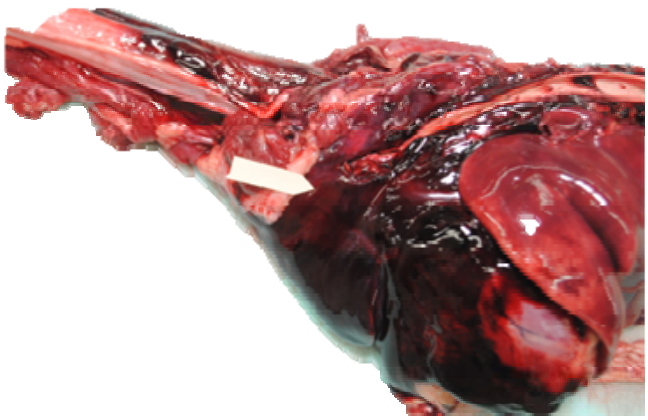

Figure 1. Acute thymic (arrow), mediastinal, and pericardial sac hemorrhage