Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

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In this issue:

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

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

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

Mailing address & contact information:
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072

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Update from the Director

The view from the Director's office

One of the major concerns we hear from our clients is regarding test results that don't match up with expectations. There are many reasons for this disparity, most of which are outlined in Dr. Tim Pasma's article in this issue: "Why didn't I get the results I expected?" AHL conducts a wide range of quality control procedures to ensure that laboratory errors are eliminated or at least minimized. These include extensive validation of each new test prior to being placed into use; incorporating positive and negative controls in each test run; double verification of transcribed results; and obtaining second opinions for qualitative diagnoses that may be challenging, unique or rare.

And yet, we are dealing with biological systems and human error. Some tests, such as PCR, are almost too sensitive and subject to contamination during the collection and testing process. Others, such as bacterial culture following antimicrobial therapy, are not sensitive enough. The best avenue to pursue when test results don't match the expected outcome is to contact the AHL veterinarian whose name is listed at the bottom of the specific laboratory test report. They are best able to troubleshoot the test and discuss the rationale for the outcome. In some cases, the decision may be made to repeat the test using the same sample or to collect a fresh sample for testing. Perhaps a second opinion is warranted for a pathologic diagnosis.

Let's work together to ensure that everyone is satisfied with the outcome of AHL laboratory testing.

Maria Spinato, Director

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

AHL New Tests Developed in 2023

Helen Oliver

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

AHL Newsletter 2024;28(1):3.

TEST METHOD	CODE	SPECIES
Cache Valley virus - PCR	cvvpcr	Caprine, Ovine
Chlamydia spp. - PCR, non-food animals	csppnf	Avian, Canine, Equine, Feline, Other
Clostridium botulinum (A, B, C, D, E, and F) toxin genes detection	bot	Avian, Bovine, Canine, Caprine, Equine, Feline, Other, Ovine, Porcine, Chicken, Turkey
Copper, biopsy - ICP-MS	tcub	Avian, Bovine, Canine, Caprine, Equine, Feline, Ovine, Porcine, Chicken, Turkey
Fish *Flavobacterium branchiophilum* - PCR (Bacterial Gill Disease)	bgdpcr	Other
Haemoplasma, *Mycoplasma haemobos* and *Mycoplasma haemolamae* - PCR	hapcr3	Bovine, Other
Influenza A virus/Equine rhinovirus A/Equine rhinovirus B - PCR	inflerv	Equine
Iodine-serum/urine - ICP-MS	iods	Avian, Bovine, Canine, Caprine, Equine, Feline, Ovine, Porcine, Chicken, Turkey
Mastitis, environmental culture - Enterobacterales	macule1	Bovine, Caprine, Ovine
Porcine sapovirus - PCR	psapopc	Porcine
S. equi subsp. *zooepidemicus* SzM - PCR	szmpcrf	Porcine
Vitamin A and E, serum - HPLC	vitae	Avian, Bovine, Canine, Caprine, Equine, Feline, Ovine, Porcine, Other
Vitamin A, serum - HPLC	vitat	Avian, Bovine, Canine, Caprine, Equine, Feline, Ovine, Porcine, Other

Equine insurance cases at AHL

Tim Pasma, Andrew Brooks

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

AHL Newsletter 2024;28(1):4.

In cases where horses need to be submitted for postmortem for insurance purposes, they do not need to be submitted live to the AHL. If euthanasia is required, **please note that only livestock weighing less than 50kg can be euthanized at AHL Guelph.** Livestock weighing greater than 50kg will be transferred to the OVC Large Animal Hospital to be euthanized by a large animal clinician and extra fees will apply.

At AHL Kemptville, euthanasia of animals weighing above 50kg is not available. Animals that exceed these weight limits or that cannot be transported humanely must be euthanized on farms.

Please refer to the AHL Fee Schedule to determine the costs for postmortem of equine insurance cases. Note that in addition to the typical postmortem and ancillary test fees, additional costs are levied for insurance/legal documentation and disposal.

Please contact ahlinfo@uoguelph.ca to review the submission process and fees for equine insurance cases. *AHL*

Why didn't I get the results I expected?

Tim Pasma

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

AHL Newsletter 2024;28(1):4.

The process for obtaining laboratory test results can be divided into 3 phases: preanalytic, analytic and postanalytic.

The **preanalytic phase** includes activities occurring before the analysis of the test, such as preparation of the patient, history, physical exam and sample collection, handling and transport. This is the phase with the largest number of influences on the sample and its test result. Studies have shown that preanalytic errors make up the largest proportion of total errors with laboratory results. Proper handling of the sample in this stage is critical, as sample type and quality can adversely affect test results. Preanalytic errors can be avoided through the use of standardized procedures and training programs for clinic staff.

The **analytic phase** is the actual testing of the sample. The AHL has a comprehensive quality assurance (QA) program in place to prevent errors in the analytic phase. Quality control checks and tests are used to ensure instruments in the lab are providing accurate results. Other QA activities include annual proficiency testing programs for technicians performing accredited tests, participation in a quarterly proficiency testing service with other laboratories, and peer-review of anatomic and clinical pathology cases at weekly rounds sessions.

However, situations sometimes arise where diagnostic test results are inconclusive or unexpected based on the history and clinical presentation of an animal. In these cases, it is important to contact the

laboratory as staff can help troubleshoot issues with the specimen or test interpretation. It may also indicate a problem with a test that warrants further investigation by the lab.

The **postanalytic phase** includes processes that occur after the sample is tested. Errors in this category include transcription errors, assigning results to the wrong patient medical record, using an incorrect reference interval, or incorrect assessment of results. Postanalytic errors can be avoided by using validation steps such as second-person verification to ensure that results are reported correctly. *AHL*

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OAHN Update – March 2024

Mike Deane, Tanya Rossi

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

It has been a busy winter for the Ontario Animal Health Network, with many completed research projects, seasonal reports, and new resources for vets. We also held our annual general meeting on February 6, which allowed everyone to get together (virtually) to hear about the activities other networks have been working on. The invited speaker was Dr. Zvonimir Poljak who presented his exciting work on the Animal Health Dashboard project. To view any of our network reports and research projects, go to OAHN.ca and navigate to the species in which you are interested.

OAHN Aquatics Network Spotlight

The OAHN Aquatics network has recently published two resources relating to the mysterious death of arctic char at the Ontario Aquaculture Research Centre. The producer-focused article is available here: Solving the Mystery: A Guide to Identifying and Treating a Recent Disease in Arctic Char; the vet-focused case study is available here: Arctic Char Case Study.

Additionally, the OAHN Aquatics network has created courses for aquaculture producers and recreational fishers on humane harvest:

- Humane Harvest for Recreational Fishers – Thinkific Course
- Humane Slaughter for Aquaculture Producers – Thinkific Course

Completed Research Projects

In the past quarter, OAHN networks concluded three research projects. Links to the summaries, full reports, and other supporting material are below:

- Identification of Culicoides species found in selected areas of Ontario from June – September 2022
    - Podcast: OAHN Bovine Podcast: Identification of Culicoides species found in select areas of Ontario with Dr. Katie Clow and Valentina Gonzalez Rodriguez
- Tracking natural varroa mite population growth in honey bee colonies
- Swine Smallholder Postmortem Project

There are many more projects nearing completion, so check back next quarter for more reports.

New Reports

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

- OAHN survey: CIRDC, leptospirosis, heartworm
- Treating acute diarrhea
- Survey: *Anaplasma* diagnosis & treatment
- Rabies update
- Update: Raw food XDR *Salmonella* Outbreak
- AM susceptibility reporting changes
- FIP treatment options

- New PCR test now available at AHL for detection of *Clostridium botulinum* toxin genes and diagnosis of botulism
- *Castellaniella*: Are we missing this bacteria in lameness cases?
- Fowl Adenovirus (FAdV, IBH), Infectious Bronchitis Virus (IBV), Infectious
- Bursal Disease Virus (IBDV) and Reovirus strains in Ontario (December 1, 2022 to Nov 30, 2023 – Q1 to Q4 2023).
- Poultry veterinary survey highlights – Q4 2023

- Strangles resources
- OAHN equine project – Selenium
- Network member reports
- Syndromic and lab surveillance dashboard
- OAHN project – *Culicoides*
- Equine research

- Interesting cases from the Animal Health Lab
- Practitioner case report: *Salmonella* Dublin outbreak in a Holstein dairy herd
- *Theileria orientalis* Ikeda in New York State
- Bovine provincial slaughter condemnation data
- Global surveillance – Bluetongue in the Netherlands
- Extensive drug-resistant *Salmonella* infections linked to raw pet food and contact with cattle

- Novel Influenza A - H3N2 Cluster 2010.1 Update
- *Salmonella* surveillance
- OAHN Veterinary clinical impression survey - veterinary comments
- Porcine Circovirus Type II (PCV2)
- Porcine Epidemic Diarrhea (PEDV) / Porcine Deltacoronavirus (PDCoV)
- International disease topics of interest summary
- OAHN project update: Porcine Hemagglutinating Encephalomyelitis virus

- January 2024 meeting summary
- Project updates
- 2023 Animal Laboratory case data
- Provincial abattoir slaughter and condemnation data
- Small ruminant resource and member updates

Insights from 16-year *Mycoplasma* culture and PCR data analysis in a Canadian provincial laboratory

Hugh Cai, Pauline Nelson-Smikle, Fernando Munevar and Staff from AHL Mycoplasmology and Molecular Biology

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

AHL Newsletter 2024;28(1):8.

For over four decades, the Animal Health Laboratory (AHL) in Ontario, Canada has been at the forefront of conducting culture isolation of *Mycoplasma* and *Ureaplasma* species. Recent advancements in laboratory techniques, including MALDI-TOF mass spectrometry (MS) and DNA sequencing, have bolstered the precision of the culture procedures. In 2007, the implementation of a Laboratory Information Management System (LIMS) further streamlined data management and facilitated trend analysis. This report provides a comprehensive summary of *Mycoplasma* culture and PCR tests at the AHL since 2007.

Over the past 16 years, the AHL has meticulously cultured a total of 18,730 samples. Interestingly, there is a trend towards declining culture samples in recent years, attributed to the growing prevalence of PCR tests (**Fig. 1**). Predominantly sourced from food animals, as detailed in (**Table 1**), the samples have led to the successful isolation of over 25 species of *Mycoplasma* and *Ureaplasma*. Key findings include the frequent identification of *M. bovis* (7%) and *M. arginini* (5%), with a noteworthy surge in *Ureaplasma diversum* during abortion outbreaks in 2019. Intriguingly, *M. bovis* was isolated only once from a semen sample since 2007, indicative of a low likelihood of transmission through semen in Ontario (**Tables 2-4**). This summary also sheds light on mollicutes (the order of cell-free bacteria including *Mycoplasma* and *Ureaplasma* species) isolated from non-food animals, highlighting prevalent species such as *M. canis* (8%), *M. edwardii* (4%), *M. cynos* (3%), *M. spumans* (2%), *Ureaplasma* sp. (2%), and *M. maculosum* (2%) (**Table 5**).

The AHL has undertaken PCR tests on 70,853 samples over 16 years, targeting 15 different *Mycoplasma* species. The data reveal a progressive increase in PCR samples annually, coinciding with a partial displacement of culture assays (**Fig. 1**). Particularly noteworthy is the significant uptick in PCR positive rates for *M. hyopneumoniae*, *M. bovis*, and *M. synoviae* in recent years. The study also draws attention to prevalent haemoplasmas, namely *M. haemofelis* and *M. haemocanis* **(Table 6**). Other haemoplasma that were identified include: *M. haemobos (1), M. haemolamae (21), M. ovis (1), M. suis (22), M. wenyonii (5), M. wenyonii* (7), Bovine haemoplasma (3), Ovine haemoplasma (2).

Significantly, PCR assays played a pivotal role in aiding the eradication of *M. iowae* in a high-value turkey breeder facility. This comprehensive overview provides valuable insights into the evolving landscape of *Mycoplasma* and *Ureaplasma* testing at the AHL, highlighting the laboratory's adaptability to cutting-edge technologies including the evolving LIMS. *AHL*

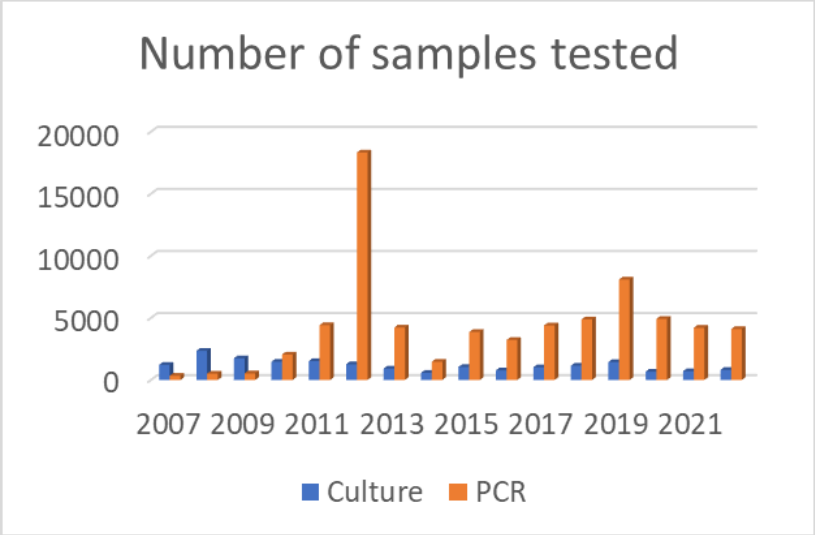

Figure 1. AHL *Mycoplasma* culture and PCR samples from May 2007 to April 2023. The PCR peaks between 2011 and 2013 were from the project of *M. iowae* eradication.

Table 1. Samples tested by Mycoplasma culture from May 2007 to April 2023.

Sample Types	Numbers
Semen	1198
Milk	5167
Food animals (excludes semen and milk)	9599
Non-food animals	2766
Total	18730

Table 2. Mycoplasma isolated from semen samples from May 2007 to April 2023.

Mycoplasma isolated	Number of samples	%
Acholeplasma laidlawii	7	0.58%
Acholeplasma oculi	3	0.25%
M. bovigenitalium	29	2.42%
M. bovis	1	0.08%
M. canadense	2	0.17%
Mycoplasma spp.	25	2.08%
Ureaplasma sp.	282	23.54%
Not Isolated	849	70.87%
Total	1198	

Table 3. Mycoplasma isolated from mastitis milk samples from May 2007 to April 2023.

Mycoplasma sp. isolated	#	%
Acholeplasma laidlawii	9	0.18
M. alkalescens	1	0.02
M. arginini	33	0.66
M. bovigenitalium	1	0.02
M. bovirhinis	1	0.02
M. bovis	111	2.21
Mycoplasma spp.	2	0.04
Not isolated	4860	96.85
Total	5018	

Table 4. *Mycoplasma* isolated from other food animal samples (non-semen and non-milk) from May 2007 to April 2023.

	Species	#	%
1	*M. bovis*	974	10.15
2	*M. arginini*	782	8.15
3	*Ureaplasma sp.*	634	6.60
4	*Acholeplasma sp.*	330	3.40
5	*M. ovipneumoniae*	314	3.27
6	*M. bovirhinis*	247	2.57
7	*M. hyorhinis*	154	1.60
8	*M. gallopavonis*	153	1.59
9	*M. bovigenitalium*	114	1.19
10	*M. hyosynoviae*	108	1.13
11	*M. iowae*	104	1.08
12	*M. alkalescens*	66	0.69
13	*M. gallinaceum*	64	0.67
14	*M. pullorum*	56	0.58
15	*Mycoplasma spp.*	49	0.50
16	*M. gallisepticum*	28	0.29
17	*M. bovoculi*	27	0.28

	Species	#	%
18	*M. gallinarum*	24	
19	*M. canadense*	21	
20	*M. conjunctivae*	20	
21	*M. synoviae*	12	
22	*M. columborale*	7	
23	*M. iners*	7	
24	*M. canis*	5	
25	*M. hyopneumoniae*	5	
26	*M. meleagridis*	3	
27	*M. capricolum*	2	
28	*M. spumans*	2	
29	*M. agalactiae*	1	
30	*M. anatis*	1	
31	*M. columbinum*	1	
32	*M. mustelae*	1	
33	Not Isolated	5282	55.03
	Total	**9599**	

Table 5. *Mycoplasma* isolated from non-food animal samples from May 2007 to April 2023

	Species	#	%		Species	#	%
1	*M. canis*	235	8.5	15	*Acholeplasma laidlawii*	3	
2	*Ureaplasma sp.*	221	8.0	16	*M. bovis*	3	
3	*M. edwardii*	103	3.7	17	*M. equirhinis*	3	
4	*M. cynos*	83	3.0	18	*M. gallinaceum*	3	
5	*M. spumans*	68	2.5	19	*M. citelli*	2	
6	*M. maculosum*	53	1.9	20	*M. gallisepticum*	2	
7	*M. felis*	45	1.6	21	*Mycoplasma* sp. HRC 689	2	
8	*Mycoplasma spp.*	41	1.5	22	*M. bovirhinis*	1	
9	*M. bovigenitalium*	20	0.7	23	*M. columborale*	1	
10	*M. molare*	17	0.6	24	*M. gallinarum*	1	
11	*M. arginine*	13	0.5	25	*M. iners*	1	
12	*M. pulmonis*	13	0.5	26	*M. leonicaptivi*	1	
13	*M. feliminutum*	7	0.3	27	*M. testudinis*	1	
14	*M. gateae*	6	0.2		Not Isolated	1817	65.7
					Total	**2766**	

Table 6. *Mycoplasma* species identified by PCR at the AHL from May 2007 to April 2023.

	# Tested	# Positive	% positive
M. hyorhinis	598	253	42.3
M. bovis	1598	482	30.2
Ureaplasma	629	187	29.7
M. synoviae	5204	961	18.5
M. hyosynoviae	730	118	16.2
M. haematoparvum	207	28	13.5
M. hyopneumoniae	15,514	2065	13.3
M. haemofelis	1157	128	11.1
M. haemominutum	363	20	5.5
M. gallisepticum	4247	230	5.4
M. haemocanis	823	40	4.9
M. iowae	38285	995	2.5
M. turicensis	363	7	1.9
M. meleagridis	926	1	0.1

Selected zoonotic pathogens and diseases from Ontario identified at the AHL in 2023

Tanya Rossi

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

AHL Newsletter 2024;28(1):12

The term 'One Health', "an integrated, unifying approach to balance and optimize the health of people, animals and the environment"[1], is a relatively new one in medicine, however the contribution of veterinarians to public health dates back 100s of years. This contribution has taken many forms, including using comparative physiology and anatomy to further human health, informing policies involving food safety and ecosystem health, and detection of zoonotic pathogens, among others. AHL participates in many of these initiatives, but our primary contribution is in the surveillance and annual reporting of zoonotic pathogens identified at our laboratory (**Tables 1 and 2**).

Case numbers for most zoonotic pathogens isolated or identified by the AHL in 2023 are relatively unchanged from the previous year, however some changes were identified. After a 4-fold increase from 2021 to 2022 Blastomyces dermatitdis cases have decreased again in canines from 13 in 2022 to 3 in 2023. Avian West Nile virus positives have increased again this year, rising from 11 in 2021 and 26 in 2022 to 50 in 2023. This increase is primarily in wild avian species. Similar increases occurred in Eastern Equine Encephalitis virus positives in equines, and in positive serology submissions for *Borrelia burgdorferi* in canines and equines. These changes in vector-borne disease may reflect changes in vector populations and distribution that should be investigated further[2,3]. Isolation of *Methicillin-resistant S. pseudintermedius* (MRSP) in canines has also increased in 2023.

The percentage of animals identified as positive for leptospirosis was roughly unchanged in 2023 in cattle, equines, and dogs, and the total number of submissions tested was about the same as 2022. The percentage of positive leptospirosis cases and total submissions tested decreased in swine. These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates. They do not take into account vaccination status, as all except horses may be routinely vaccinated for leptospirosis. Monitoring programs are not included. *Brucella canis* results shown are positive on the 2ME-RSAT.

Table 1. Number of cases for selected zoonotic pathogens isolated and/or identified at the AHL, 2023.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2023	2022
Ascarids (incl *T. canis, T. cati, T. leonina, Baylisascaris* sp.)	1	11	5			44	2	18	5	7	**93**	**110**
Blastomyces dermatitidis								3			**3**	**13**
Bordetella bronchiseptica		33	3				1	8	1	3	**49**	**51**
Borrelia burgdorferi (Lyme disease), serology			19					17	1		**37**	**16**

Brucella sp. (non-*abortus)*												**0**	**0**
Campylobacter coli/ jejuni/ fetus subsp. *fetus*				2				3	2			**7**	**15**
Chlamydia sp.				5	10					1		**16**	**24**
Clostridium difficile		1						2				**3**	**2**
Coxiella burnetii (Q fever)	12			15	18							**45**	**56**
Cryptococcus sp.												**0**	**3**
Cryptosporidium sp.	122			3	4					11		**140**	**193**
Eastern equine encephalitis virus			10									**10**	**3**
Echinococcus multilocularis								15				**15**	**7**
Giardia sp.	10							24	7	4		**45**	**40**
Listeria monocytogenes	4	1		7	6					1		**19**	**25**
Methicillin-resistant *Staph aureus* (MRSA)			2							1		**3**	**7**
Methicillin-resistant *S. pseudintermedius* (MRSP)			1					87	2	7		**97**	**55**
Rabies virus												**0**	**0**
Salmonella enterica	40	1				28	4			8		**41**	**29**
Streptococcus suis	3	125	1			3				3		**135**	**204**
Streptococcus equisimilis		39	15		1			1		1		**57**	**72**
Streptococcus zooepidemicus	1	3	177		1			2	2	1		**187**	**157**
Toxoplasma sp.				9	2				2			**13**	**18**
Verotoxigenic *E.coli* (VTEC)	2											**2**	**3**
West Nile virus			5							45		**50**	**27**
Yersinia enterocolitica	2											**2**	**2**
Total												1433	1433

Table 2. *Leptospira* spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL, 2023.

Leptospira spp. serovar	**Bovine**	**Swine**	**Equine**	**Canine**	**Other**
L. autumnalis	21	0	22	67	1
L. bratislava	26	1	25	34	0
L. canicola	28	0	6	46	0
L. grippotyphosa	9	0	1	37	0
L. hardjo	42	1	9	11	0
L. icterohaemorrhagiae	40	1	14	61	0
L. pomona	37	1	10	47	0
IHC or PCR-positive	0	0	0	4	0
Positive/tested cases	**55/198**	**1/14**	**30/45**	**88/173**	**1/12**
% pos	27.8%	7.1%	66.7%	50.9%	8.3%
% pos, 2023/2022	28/30%	7/21%	67/67%	51/51%	8/0%

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RUMINANTS

A retrospective analysis of 10 years' submissions of small ruminant abortions (2014-2023)

Amanda Mansz, Maria Spinato

Animal Health Laboratory, University of Guelph, Guelph, ON

AHL Newsletter 2024;28(1):15.

Over the past 10 years at the Animal Health Lab, AHL and Pathobiology pathologists have completed 473 ovine abortion cases and 353 caprine abortion cases. Diagnostic investigation of small ruminant (SR) abortions often requires a full diagnostic work-up to determine a cause. A routine SR abortion investigation begins with gross postmortem of one or multiple fetuses with placenta, histopathology, bacterial culture of fetal lung, fetal abomasal content and placental cotyledons, and a PCR panel on placenta for detection of *Toxoplasma gondii*, *Coxiella burnetii* and *Chlamydia abortus*. If one of these tests is omitted, interpretation of the remainder of the tests can be challenging. For example, if placental tissue tests PCR positive for the detection of *Toxoplasma* or *Coxiella* and histopathology is not performed, it is difficult to confirm whether this abortion is truly due to infection with one of these organisms, or if the PCR has detected a placental contaminant (i.e., pathogen is present in the flock/environment, but is not the cause for abortion in that case). Bacterial culture without histopathology leads to a similar conundrum. If a full range of tests is not possible, a publication by Hazlett et al. provides cut-point guidelines for quantitative PCR Ct values in relation to agents deemed to be the cause of abortion (rather than detection without further evidence of causation): https://journals.sagepub.com/doi/pdf/10.1177/1040638713484729

The top three infectious causes for both ovine and caprine abortion cases every year from 2014 - 2023 were *Chlamydia abortus*, *Coxiella burnetii* and *Toxoplasma gondii* (**Fig. 1**).

A summary of the most commonly implicated causes in each species is provided in (**Fig. 2**). Graphs of 10-year trends of the most common causes of abortion for both sheep and goats are provided in (**Fig. 3**).

In general, the vast majority of the cases diagnosed as idiopathic abortion lacked a full abortion workup as detailed above. Included in this category of "idiopathic abortions" are abortions considered to be non-infectious (i.e., abortion due to multiple births/suspected dystocia), and a small proportion of cases in which a full abortion workup was completed, and no conclusive diagnosis could be made despite additional testing.

The importance of SR abortion investigation is not only essential for flock health and production success, but also can be significant for human health, as many of these abortifacient infectious agents are considered zoonotic. *Coxiella burnetii*, *Chlamydia abortus*, *Listeria monocytogenes* and even Cache Valley virus (CVV) all have the potential to cause illness in people. AHL is now pleased to offer in-house CVV PCR testing to confirm the latter etiology. Note that a negative PCR result does not rule out CVV as a possible etiology, as the virus may be cleared during gestation and will therefore be absent at the time of birth/abortion. In cases where CVV is strongly suspected, and the PCR is negative, fetal thoracic cavity fluid can be referred to another laboratory for serologic confirmation.

Over the past ten years, the AHL has published several newsletter articles highlighting interesting, emerging, and atypical cases of SR abortions. A list of these newsletter articles and links are provided in a summary at the end of this article. *AHL*

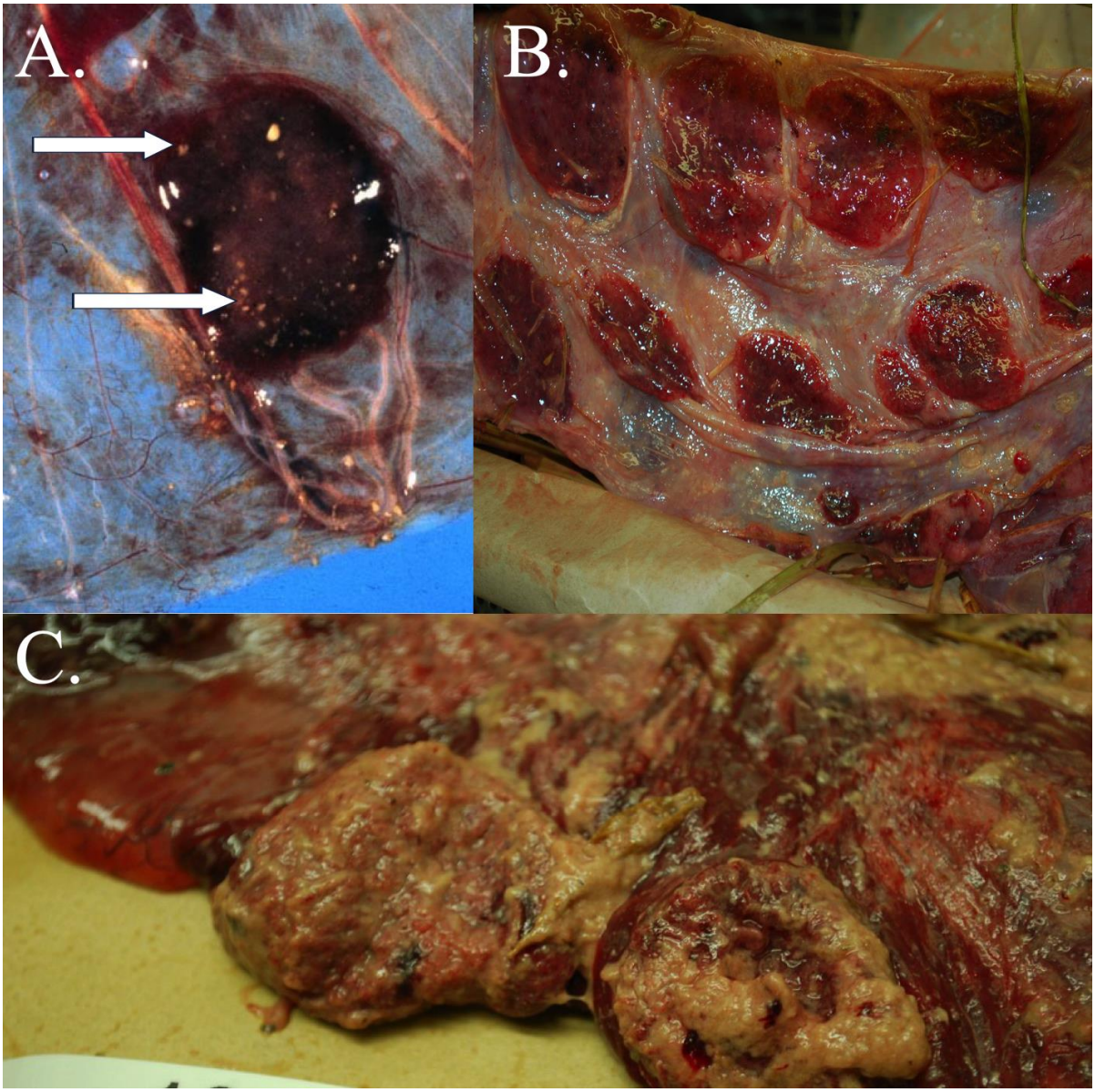


Figure 1: Small ruminant placentitis. **A.** *Toxoplasma* placentitis with classical discrete foci of necrosis and mineralization of the cotyledon (arrows). **B.** *Coxiella* placentitis with thickened opaque intercotyledonary spaces. **C.** *Chlamydia* placentitis with abundant inflammatory exudate and necrosis involving cotyledons and intercotyledonary spaces.

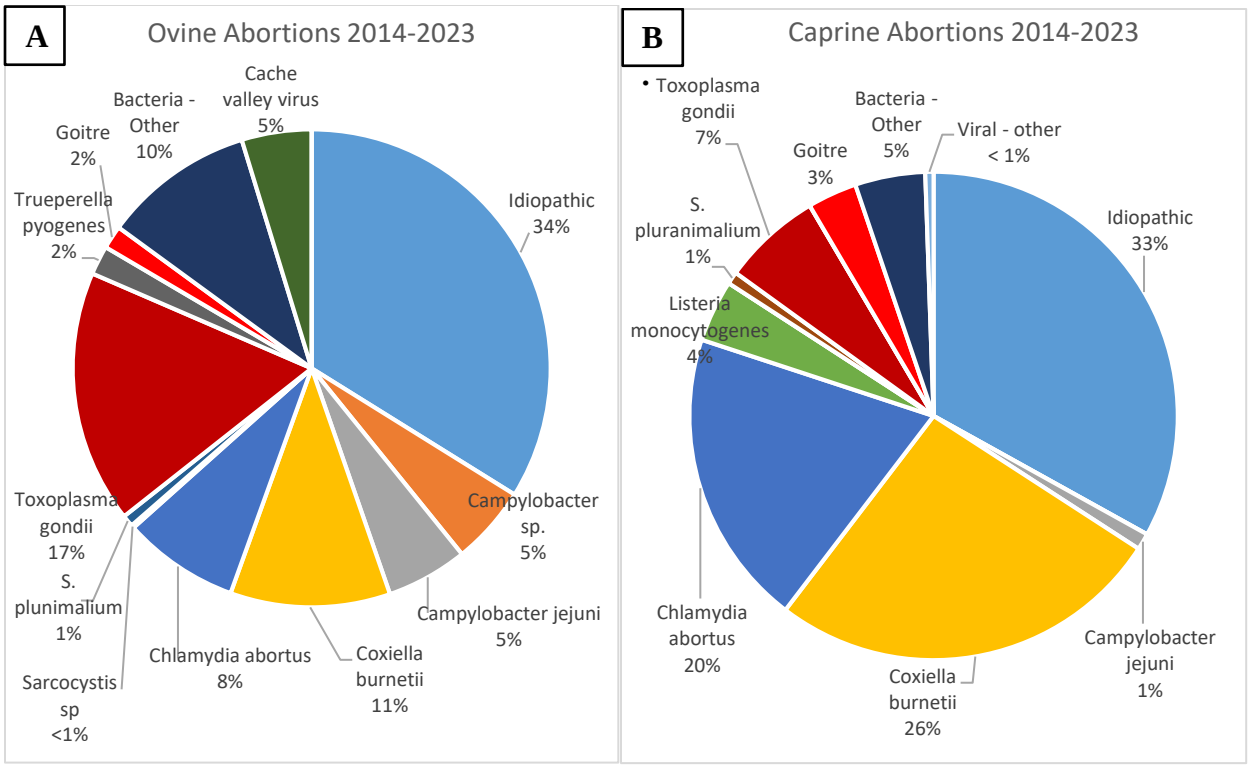
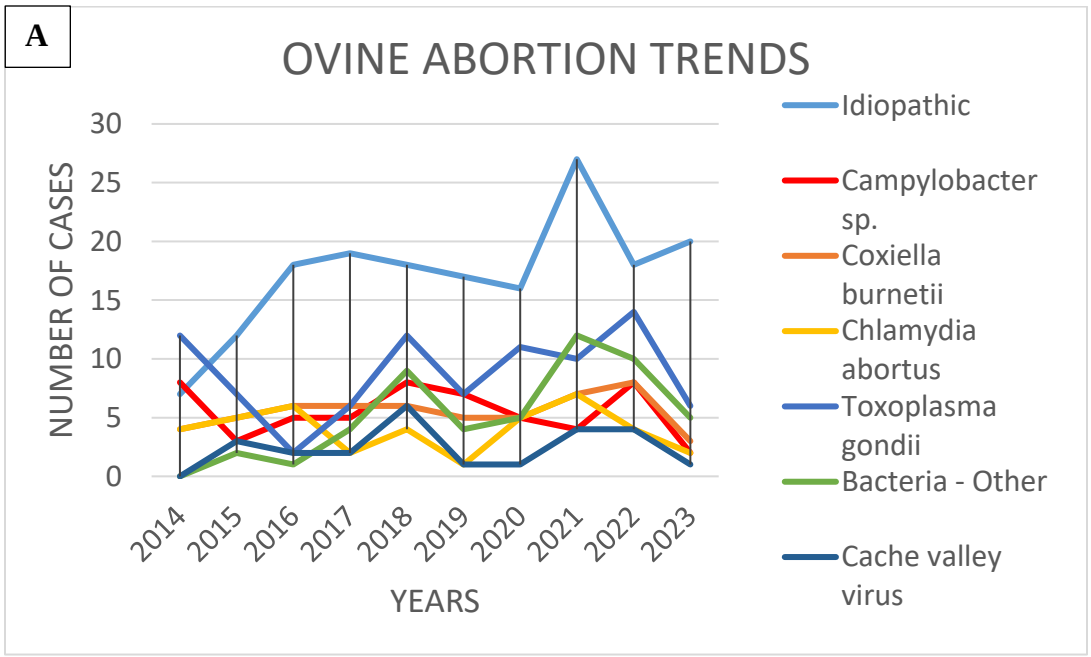


Figure 2. A ten-year compilation (2014-2023) of the causes of small ruminant abortions at the AHL. **A.** Ovine abortions **B.** Caprine abortions



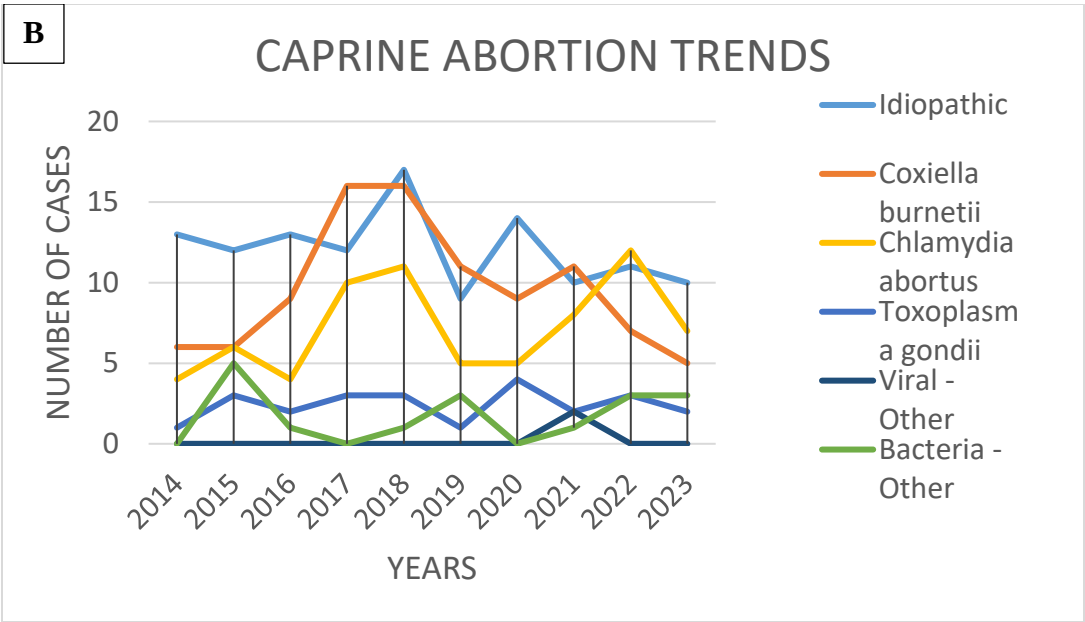


Figure 3. Ten-year trends (2014-2013) of the most frequent causes of small ruminant abortions. **A.** Ovine abortions **B.** Caprine abortions

A Summary of AHL Small Ruminant Abortion newsletter articles over the past 10 years:

March 2016
Cache Valley virus – an outbreak of congenital malformations in Ontario lambs
https://www.uoguelph.ca/ahl/content/ruminants-1

December 2017
Attention small ruminant practitioners! Suspected Cache Valley virus abortions in southern Ontario
https://www.uoguelph.ca/ahl/content/ruminants-13

June 2019
Abortion caused by *Helicobacter trogontum* in a sheep flock
https://www.uoguelph.ca/ahl/content/ruminants-20

September 2020
Campylobacter spp. abortion in ruminants
https://www.uoguelph.ca/ahl/campylobacter-spp-abortion-ruminants

March 2021
Ovine abortion with fetal congenital anomalies – Cache Valley virus (CVV)
https://www.uoguelph.ca/ahl/ovine-abortion-fetal-congenital-anomalies-–-cache-valley-virus-cvv

March 2022
Cache Valley virus abortions in goat kids
https://www.uoguelph.ca/ahl/cache-valley-virus-abortions-goat-kids

December 2022
Ovine abortion due to *Actinobacillus seminis* and *Histophilus somni*
https://www.uoguelph.ca/ahl/ovine-abortion-due-actinobacillus-seminis-and-histophilus-somni

June 2023
Yersinia pseudotuberculosis abortions in small ruminants
https://www.uoguelph.ca/ahl/yersinia-pseudotuberculosis-abortions-small-ruminants

Reference
1. Hazlett MJ, et al. A prospective study of sheep and goat abortion using real-time polymerase chain reaction and cut point estimation shows *Coxiella burnetii* and *Chlamydophila abortus* infection concurrently with other major pathogens. J Vet Diagn Invest. 2013;25(3):359-68.

The bovine abortion PCR panel: A six-year summary

Emily Brouwer, Davor Ojkic

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

AHL Newsletter 2024;28(1):19.

The bovine abortion PCR panel was made available in 2018 as a means to bundle testing for abortogenic pathogens in bovine specimens. This PCR detects bovine herpesvirus-1 (BoHV-1), *Leptospira* spp., and *Neospora caninum,* and can be performed on fetal tissues (kidney, liver spleen) or placenta. In cases where only formalin-fixed paraffin-embedded tissue is available, the PCR can also be performed on scrolls of tissue blocks. This test is routinely included in the diagnostic workup when fetuses are submitted to the lab or can be ordered when submitting specimens from field postmortems.

Between the introduction of the PCR in 2018 and the end of 2023, a total of 887 bovine abortion PCR panels have been performed. Overall, BoHV-1 has been detected in 5.02% of the submitted tests, *Leptospira* spp. has been detected in 1.72% of submissions, and *Neospora caninum* has been detected in 6.53% of submissions (**Tables 1, 2, 3)**.

Table 1. Summary of bovine abortion panel PCR results for bovine herpesvirus-1 (2018-2023).

BHV-1 result	2018	2019	2020	2021	2022	2023
Negative	169	141	129	159	100	145
Positive	7	8	4	9	7	9
Total	176	149	133	168	107	154
	3.98%	5.37%	3.01%	5.36%	6.54%	5.84%

Table 2. Summary of bovine abortion panel PCR results for *Leptospira* spp. (2018-2023).

Leptospira result	2018	2019	2020	2021	2022	2023
Negative	172	148	129	166	105	152
Positive	4	1	4	2	2	2
Total	176	149	133	168	107	154
	2.27%	0.67%	3.01%	1.19%	1.87%	1.30%

Table 3. Summary of bovine abortion panel PCR results for *Neospora caninum* (2018-2023).

Neospora caninum result	2018	2019	2020	2021	2022	2023
Inconclusive		1		1	3	
Negative	170	131	121	162	100	147
POSITIVE	6	17	12	5	4	7
Grand Total	176	149	133	168	107	154
	3.41%	12.08%	9.02%	3.57%	6.54%	4.55%

When bovine abortion cases are submitted to the Animal Health Laboratory, the bovine abortion PCR panel is typically run in conjunction with other ancillary testing, including postmortem examination, histology, bacterial culture, other PCRs (BVDV, *Ureaplasma*), and *Leptospira* microagglutination testing. When sent in from external clients, this panel is often submitted as a stand-alone test, which can lead to some challenges in interpretation as detection of an agent does not necessarily constitute an etiologic diagnosis.

In cases of BoHV-1, it was noted that when histology was performed in conjunction with the PCR test, almost all positive cases had characteristic histologic lesions. In one case where BoHV-1 was detected with a relatively high cycle threshold, there were no characteristic herpesviral lesions on microscopic examination, and abortion was attributed to bacterial infection. In this case, the PCR was repeated on fetal liver and was negative.

Diagnosis of abortion due to leptospirosis proved to be more challenging. Although detected by PCR, many cases did not have characteristic histologic lesions, or had non-specific lesions and required follow-up testing. In most cases where *Leptospira* spp. was detected, AHL pathologists were able to perform additional testing (*Leptospira* microagglutination (MAT) testing and immunohistochemistry (IHC)) to further investigate the potential contribution of this pathogen to abortion. In cases where *Leptospira* infection was determined to be the cause of abortion, the diagnosis was usually confirmed with IHC or MAT. Of all the positive cases, only one test was submitted as a send-in, and no confirmatory testing was performed.

In cases where *Neospora caninum* was detected, most cases where histology was available had either characteristic or suggestive microscopic lesions. Rarely, histology was not performed due to the state of preservation of the fetus. Two send-in cases included tissues for histology as well; one of which had characteristic histologic lesions, and the other only included placenta with no fetal tissues. In rare cases where the PCR was inconclusive, pathologists were able to confirm the diagnosis either with routine histology, IHC, or *Neospora caninum* ELISA.

The bovine abortion PCR panel has become an integral part of the work up for bovine pregnancy loss. We encourage veterinarians investigating abortions in bovine herds to include formalin-fixed tissues for microscopic examination, as well as various fresh fetal tissues and maternal serum for additional testing (if indicated). The Animal Health Laboratory User's Guide and Fee Schedule provides sampling advice, if needed. *AHL*

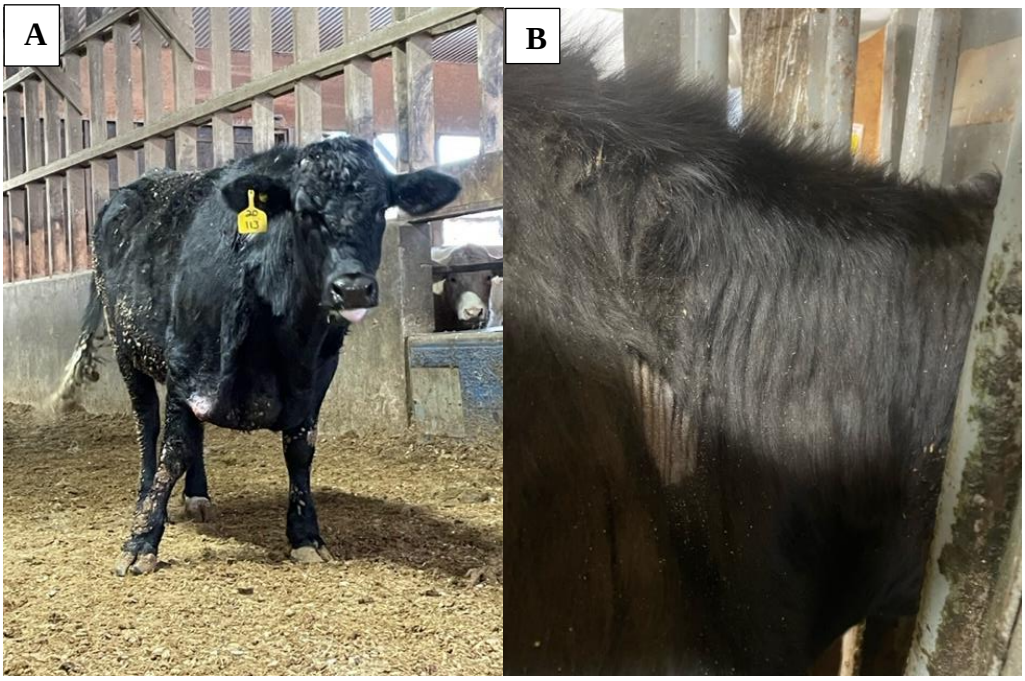
Presumptive dermatosparaxis in a beef steer

Emily Brouwer, Emily zur Linden

Animal Health Laboratory, University of Guelph, Guelph, ON (Brouwer), Metzger Veterinary Services, Linwood, ON (zur Linden).

AHL Newsletter 2024;28(1):21.

In early December, a feedlot producer called the veterinarian to examine a 1300 lb. Holstein-Angus cross steer with a large lump on the right shoulder. It was noted at the time of examination that this mass was a large fluid-filled sac (seroma). Upon further examination, it was noted that the steer had excessive skin on the forehead, and loose folds of skin over the body (**Fig. 1)**. Upon manipulation, the skin was noted to be excessively elastic and pulled away from the body and face easily. The veterinarian suspected bovine dermatosparaxis and took several skin biopsies of this steer and an age-matched control and submitted them to the Animal Health Laboratory for microscopic examination.



Figures 1. Affected steer with excessive loose folds of skin. A. Excessive loose skin on the forehead and right brisket region. B. Left side of the neck with highly folded skin and focus of erosion.

In all sections of skin, the epidermis is mildly hyperplastic, hyperkeratotic, and has a thickened basement membrane. There is a subtle increase in loose basophilic ground substance surrounding slightly thinner and haphazardly arranged superficial dermal collagen bundles and superficial dermal vessels. There are clustered eosinophils, lymphocytes and plasma cells surrounding superficial dermal vessels (**Fig. 2)**. Collagen bundles in the deep dermis are histologically unremarkable but are widely separated and surrounded by thinner bands of collagen. Deep dermal blood vessels often have thickened vascular walls, with occasional lamellar mural fibrosis.

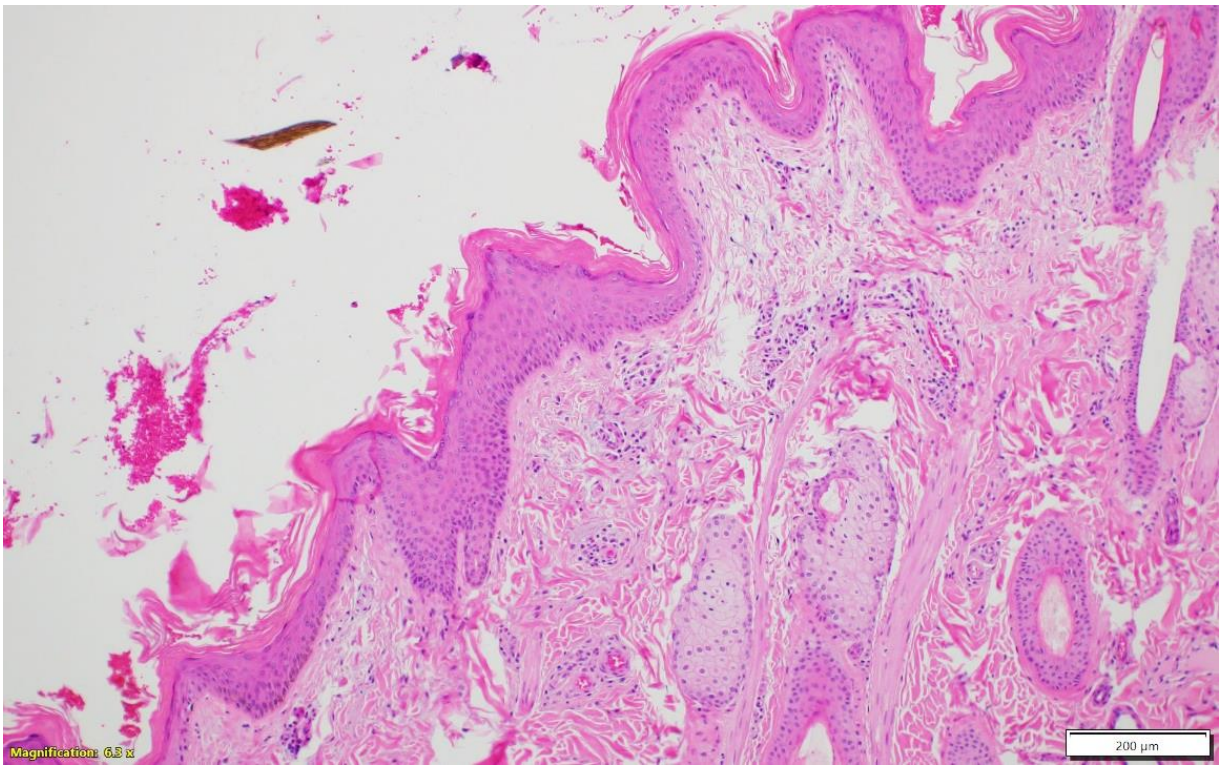
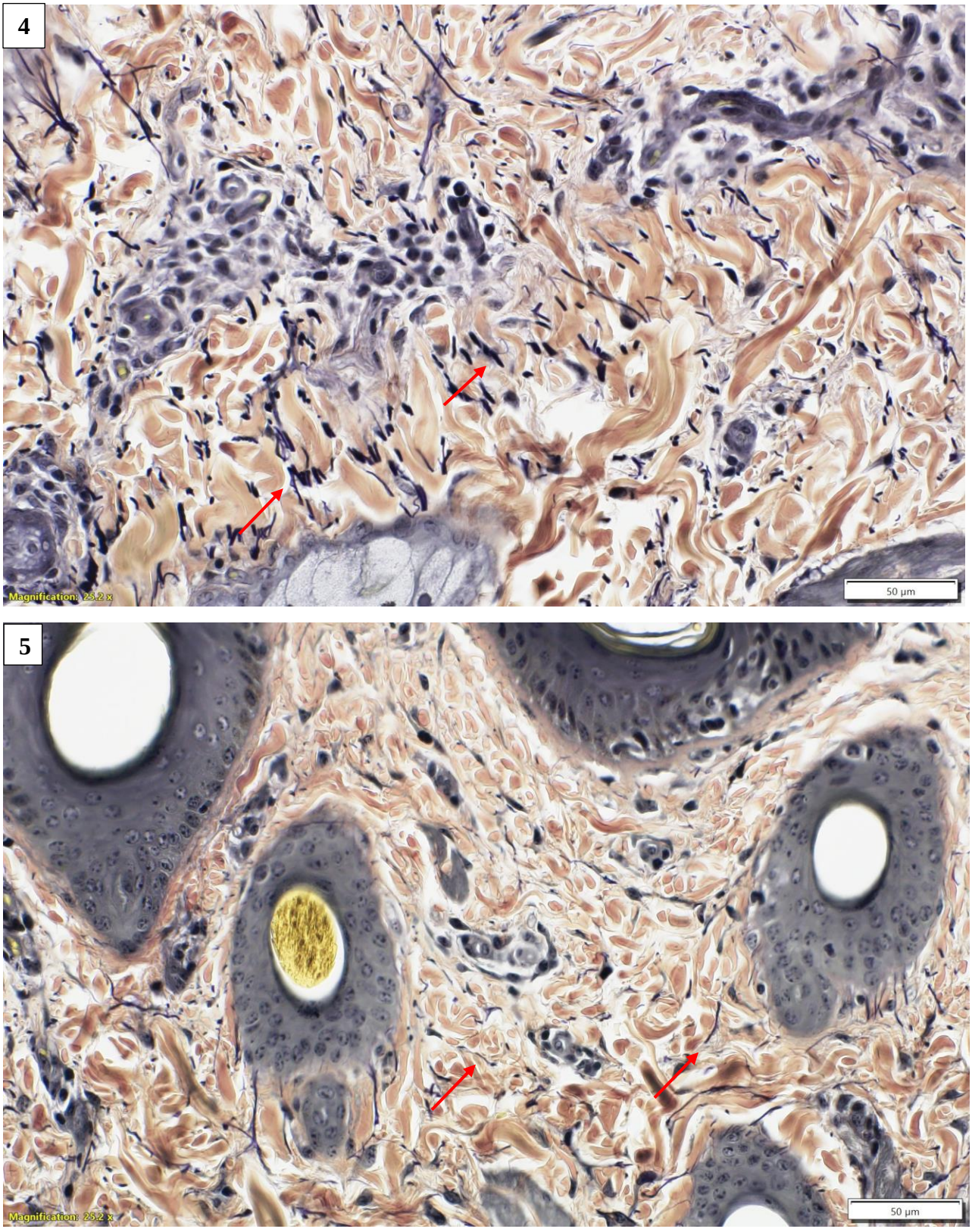


Figure 2. Affected skin, Hematoxylin and Eosin stain, 10X. The epidermis is mildly hyperplastic, hyperkeratotic and has a thickened basement membrane. There is increased dermal ground substance and thin superficial dermal collagen bundles.

The age- and breed-matched control skin provided with the samples was determined to be histologically normal and was used as a control for a Verhoeff (elastin) stain. Comparing the two elastin-stained sections, it was noted that elastin fibres in the skin of the affected steer were mildly increased in number and appeared thickened, truncated, and haphazardly arranged (**Fig. 4**) relative to the control (**Fig. 5)**. Based on these findings, a histologic diagnosis of collagen dysplasia was made.



Figures 4 and 5. Affected skin (top) and control skin (bottom), Verhoeff elastin stain, 40X magnification. Elastin fibres (red arrows) are increased in number, thickened, and truncated compared to control.

Further characterization of the collagen defect would require both ultrastructural examination (electron microscopy), and genetic testing. Electron microscopy was not pursued, and there is no commercially available genetic test for bovine dermatosparaxis. Since this animal was a feedlot steer and would not contribute to further herd genetics, additional confirmatory testing was not pursued.

Dermatosparaxis is an uncommon connective tissue disorder that is sporadically reported in cattle. Analogous to the human condition of Ehlers-Danlos Syndrome type VII C, this condition is characterized by excessive loose folds of skin, skin hyperextensibility and fragility, slow or aberrant wound healing, and excessive bruising. The disorder is the result of a mutation in the gene for procollagen I N-proteinase which is the enzyme responsible for processing types I and II procollagen, resulting in accumulation of abnormal precursor molecules and inhibition of collagen cross-linking. Ultimately, this condition culminates in the inability to produce mature collagen.

In Belgian Blue cattle, dermatosparaxis has been associated with a deletion or substitution resulting in a premature stop codon in the ADAMTS2 gene. Reports of this condition in other breeds of cattle, including Draksensberger cattle in South Africa, and Limousin calves in Ireland, have not identified this specific mutation. *AHL*

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2. Holm, DE, et al. The occurrence of dermatosparaxis in a commercial Draksensberger cattle herd in South Africa. J S Afr Vet Assoc 2008;79(1):19-24.
3. Mauldin EA, Peters-Kennedy J. Chapter 6: Integumentary System. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, 6th ed. Maxie, MG, ed. Elsevier, 2016; vol 1:542-544.

Update on porcine sapovirus detection in Ontario swine

Davor Ojkic, Josepha DeLay

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

AHL Newsletter 2024;28(1):25.

In-house PCR testing for porcine sapovirus (PSaV) has been available at the AHL since September 2023. Results to date from Ontario herds are similar to those described in other geographic areas in North America, with PSaV-associated diarrhea most frequently identified in nursing piglets around 10 days of age, often associated with rotavirus co-infection.

Between September 2023 and February 2024, a total of 83 samples from 32 Ontario herds were tested by PCR for PSaV. Of these, PaSV nucleic was detected in 30 samples from 16 herds. The age range of PSaV-positive pigs was 3-28 days, with an average age of 9 days. Histopathology was carried out for 13 of the 16 PSaV-positive herds, and atrophic enteritis typical of viral enteritis was identified in 12 of the 13 herds (**Fig. 1**). *In situ* hybridization (ISH) for PSaV in 2 herds confirmed viral presence within intestinal lesions (**Fig. 2**). Rotavirus PCR was carried out on each of the PaSV PCR-positive samples, and 17/30 (57%) samples had concurrent infection with rotavirus A, B, and / or C. PCR for porcine enteric coronaviruses (PEDV, TGEV, PDCoV) was carried out on 9 of the PSV-positive samples that were also tested for porcine rotavirus, and coronaviruses were not detected in any of the samples.

These findings reinforce the importance of testing for multiple viral pathogens in nursing and some older pigs with diarrhea. Although the full significance of PSaV to the Ontario swine herd remains to be determined, current information indicates that the virus does contribute to diarrhea predominately in neonatal pigs, but also occasionally in older nursing or weaning-aged pigs. *AHL*

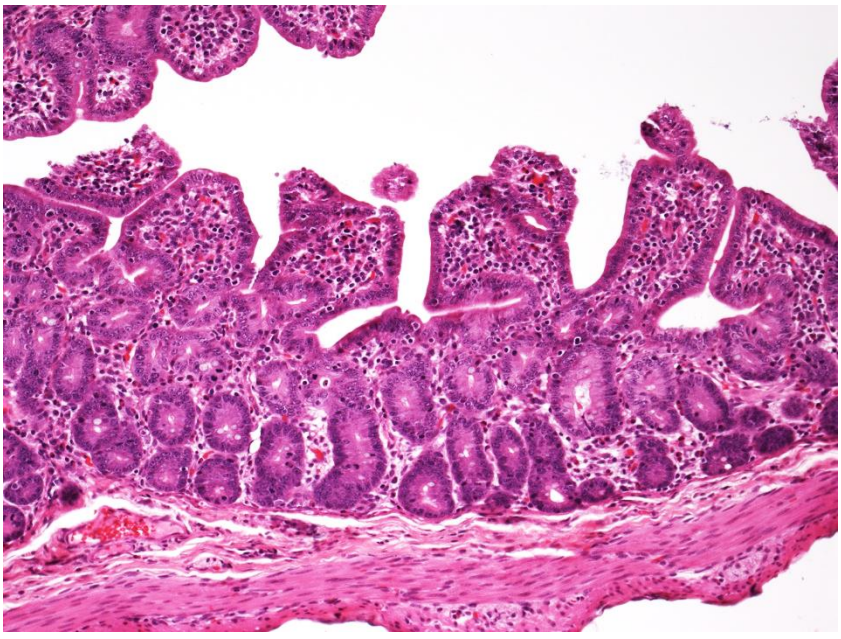

Figure 1. Atrophic enteritis in a 21-day old pig. Villi are short and blunted, and cuboidal to jumbled epithelium covers villus tips. PSaV nucleic acid was detected in feces by PCR (Ct 16.82). H&E stain.

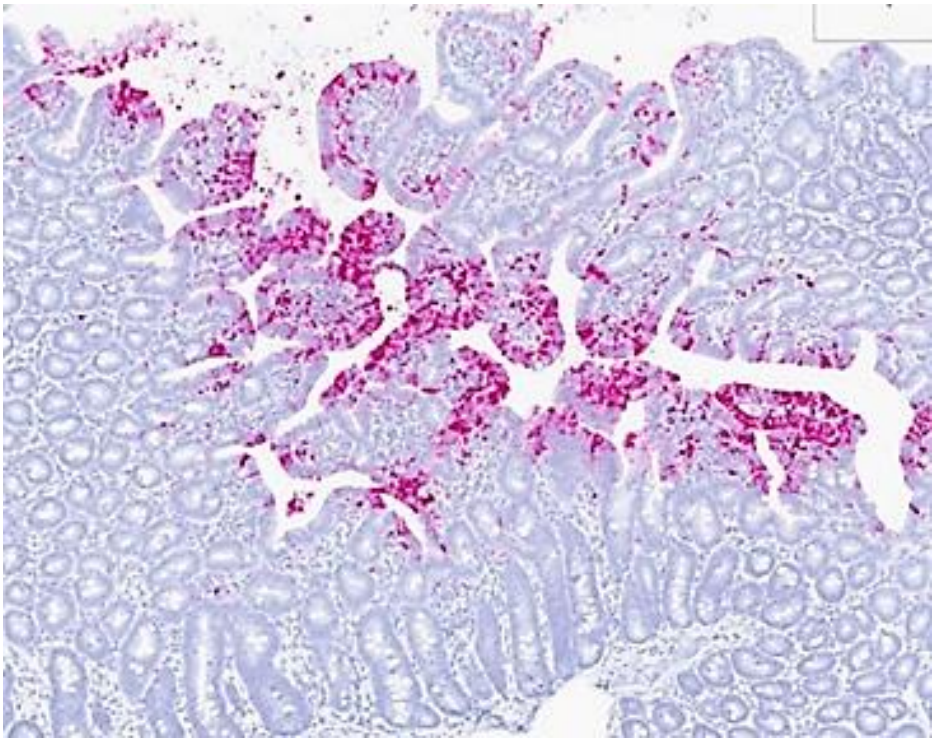

Figure 2. PSaV RNAscope (*in situ* hybridization), small intestine of same pig as in Figure 1. PSaV nuclei acid (pink chromogen) is detected in small intestinal mucosa, predominately in epithelial cells at villus tips and in lamina propria. Although porcine rotavirus nucleic acid was also detected by PCR in feces from this group, rotavirus antigen was not detected in association with histologic lesions of atrophic enteritis. Taken together, these results support PSaV as the primary etiology for atrophic enteritis and diarrhea in this group. Photo courtesy of Dr. R. Derscheid, Iowa State University.

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1. Nagai M, et al. Porcine sapoviruses: Pathogenesis, epidemiology, genetic diversity, and diagnosis. Virus Res 2020; https://doi.org/10.1016/j.viruses.2020.198025
2. Shen H, et al. Genetic characterization of porcine sapoviruses identified from pigs during a diarrhea outbreak in Iowa, 2019. Transbound Emerg Dis 2022;69:1246-1255.
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Necrotizing pancreatitis in guinea fowl associated with adenovirus infection

Andrew Brooks, Emily Martin, Alex Weisz

Animal Health Laboratory, University of Guelph, Guelph, ON; Smith and Weisz Poultry Veterinary Services, Kitchener, ON (Weisz).

AHL Newsletter 2024;28(1):27.

In January 2024, the AHL received a submission from a flock of guinea fowl experiencing elevated mortality. The mortality was affecting younger birds approximately 1-2 weeks of age. The on-farm postmortem examinations revealed gross lesions of necrosis and inflammation in the pancreatic tissues of the affected birds.

Histologically, all sections of pancreas exhibited severe, multifocal to generalized necrosis, with interstitial inflammation (**Fig. 1A**). There was also atrophy and loss of the pancreatic parenchyma. In some sections of pancreas, the necrosis was confluent. There were exudates of fibrin on the pancreatic surface and in some sections, there was generalized inflammation with infiltrates of lymphocytes and plasma cells expanding the interstitium. Throughout all of the pancreatic lesions, there were numerous large amphophilic intranuclear inclusion bodies consistent with adenovirus (**Fig. 1B**).

In guinea fowl, fowl aviadenovirus infections have been associated with erosions in the gizzard, ventriculitis and pancreatitis, and outbreaks of necrotizing pancreatitis have been reported in young guinea poults. A recent investigation of outbreaks of mortality and pancreatitis in guinea fowl flocks in Europe identified fowl adenovirus type 1 (FAdV-1) as the etiologic agent. In that investigation, clinical disease and mortality were highest in younger birds between 1-3 weeks of age, and mortality reached as high as 10-25%. The diagnosis of this disease in guinea fowl is based on the gross and histological lesions, including characteristic inclusions in the pancreas, as well as detection of FAdV-1 by PCR. *AHL*

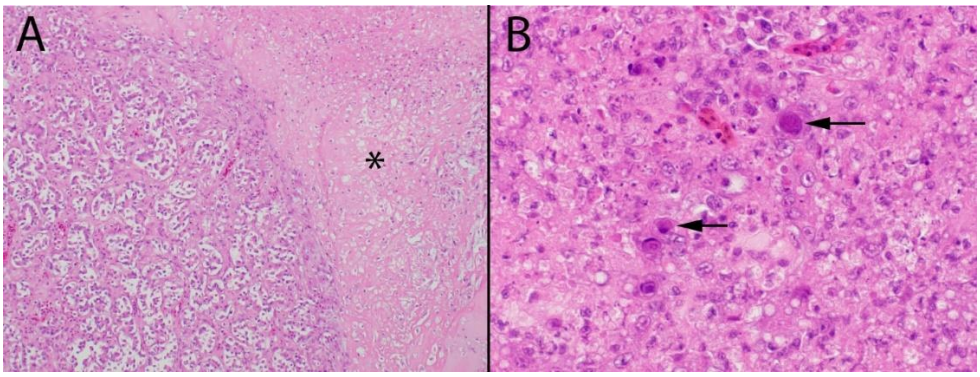

Figure 1. Lesions in the pancreas of guinea fowl associated with adenovirus infection. **(A)** There is extensive pancreatic necrosis (*) with inflammation, atrophy and loss of parenchyma. **(B)** There are numerous intranuclear inclusion bodies consistent with adenovirus (arrows). H&E stain.

Reference

1. Croville G, et al. Detection and typing of a Fowl Adenovirus Type 1 agent of pancreatitis in guinea fowl. Avian Dis 2021; 65(3):429-437.

Gangrenous dermatitis in commercial poultry

Emily Martin and Emily Brouwer

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

AHL Newsletter 2024;28(1):28.

In the fall of 2023, the AHL received multiple cases of broiler breeder chickens with increased mortality and skin lesions. The skin changes varied from mild congestion and subcutaneous edema to marked red-purple discolouration of the skin, edema, and crepitus (**Fig. 1**). Bacterial culture of subcutaneous swabs identified various combinations of *C. septicum*, *C. perfringens*, *Staphylococcus aureus*, *E. coli* and *E. cecorum*. The presence of *Clostridium* sp. was consistent with a diagnosis of gangrenous dermatitis.

Gangrenous dermatitis can occur in both chickens (4-5 weeks of age) and turkeys (13-16 weeks of age) that are approaching market age. The economic impact occurs due to increased mortality, increased condemnations, and carcass downgrading. Mortality is usually 1-5% higher in affected flocks compared to unaffected flocks, but can occasionally exceed this level (i.e., up to 60%).

Flocks present with a sudden increase in mortality. Clinically, the birds are depressed, off feed, weak, ataxic and laterally recumbent. The most significant locations for skin lesions include the breast, back, abdomen, thighs, wings, and tail (turkeys). The skin lacks feathers can be dry or moist and is discoloured dark red or green. Affected skin can have a range of lesions including edema, emphysema, and multifocal to coalescing hemorrhages that can extend into the underlying musculature. Carcasses rapidly autolyze. Microscopically, necrosis primarily occurs in the epidermis and dermis but can extend into the subcutaneous tissues and underlying skeletal muscle (**Fig. 2**). The subcutaneous tissues have accumulation of serofibrinous exudate and emphysema. There are Gram-positive rods noted throughout all skin layers and muscle with minimal inflammatory response.

The primary bacteria involved are either *C. septicum*, *C. perfringens* type A, or both. These bacteria produce toxins resulting in the tissue necrosis described. Numerous other aerobic or anaerobic bacteria can be isolated in combination with these clostridial species.

Environmental conditions and immunosuppression can interact to predispose to development of this disease. Environmental conditions include skin lesions (i.e., trauma due to cannibalism or fighting), stocking density, wet litter, poor ventilation, water leaks, and contamination (i.e., feed, water, equipment, vaccines). Immunosuppressive agents include IBDV, CAV, reovirus, IBHV, and HEV (turkeys).

The pathogenesis is not well understood and there are two theories for the introduction of bacteria. The first theory is skin trauma with external seeding of bacteria. The second is intestinal overgrowth of bacteria, loss of intestinal integrity, and systemic distribution of bacteria. Potential rule outs include contact dermatitis, mycotic dermatitis, bacterial cellulitis (non-clostridial), scabby hip (broilers) and focal ulcerative dermatitis (turkeys).

A presumptive diagnosis can be based on clinical signs, postmortem lesions, and histopathology. Subcutaneous swabs for aerobic and anerobic culture can be used to confirm this diagnosis. *AHL*

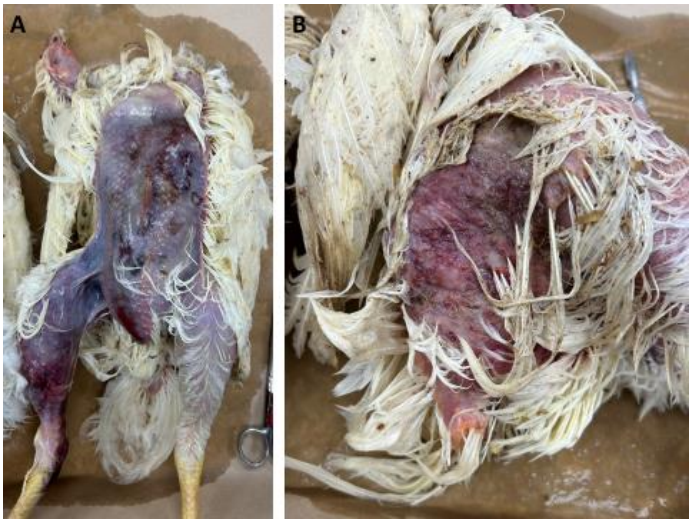

Figure 1. Skin lesions suggestive of gangrenous dermatitis (A: Breast. B: Lateral thigh)

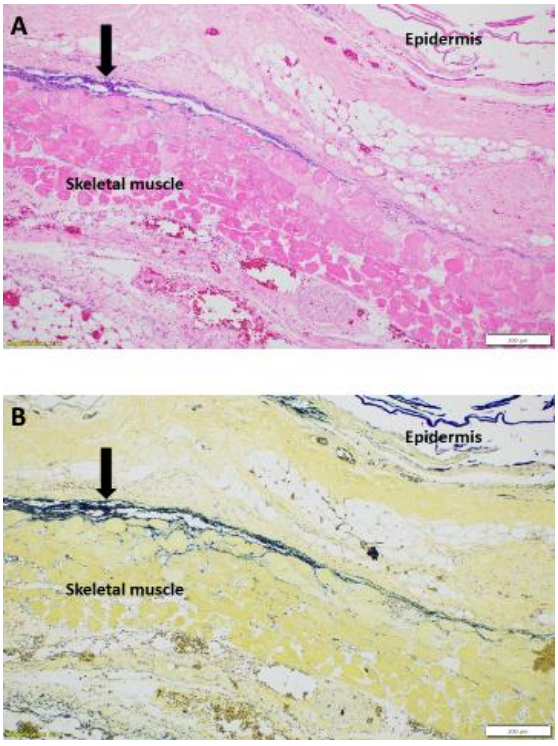

Figure 2. Histologic lesions of gangrenous dermatitis.
A: H&E stain. Necrosis and edema of dermis, subcutis and muscle including a narrow purple line of bacterial growth (arrow).
B: Gram stain showing blue staining of Gram-positive rod bacteria consistent with clostridial species (arrow).

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2.Li G, et at. An outbreak of gangrenous dermatitis in commercial broiler breeders. Avian Pathology 2010;39:247-253.
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Carbamate poisoning in wildlife

Felipe Reggeti, Neil Pople, Nick Schrier

Animal Health Laboratory, University of Guelph, Guelph, ON (Reggeti, Schrier); Veterinary Diagnostic Services, Manitoba Agriculture (Pople)

AHL Newsletter 2024;28(1):30.

Two bald eagles and a red fox were found dead on a farm. The landowner indicated that more dead animals had been found in the same area but did not specify the species or the number of animals. It was suspected that someone had been shooting wildlife from the road or poisoning the animals; therefore, the case was reported to conservation officers who investigated the site and collected the carcasses.

The carcasses were submitted to Veterinary Diagnostic Services, Manitoba Agriculture for diagnostic work-up. Gunshot trauma was ruled out. The fox was scavenged, but there were no other significant findings on postmortem examination. As part of ongoing surveillance, the eagles and fox were tested for highly pathogenic avian influenza virus (HPAI) and were negative. Liver samples were submitted to the toxicology section of the Animal Health Laboratory, University of Guelph for analysis. Carbofuran and 3-hydroxy-carbofuran were detected by LC-MS/MS, confirming exposure.

Carbofuran (trade name Furadan) is a systemic carbamate pesticide for the control of insects (direct contact and/or ingestion) on fruits and vegetable crops. It is highly toxic to humans and animals via oral exposure (LD_{50}: 5 mg/kg rat; 2 mg/kg mice; 0.48 to 0.51 mg/kg in mallard ducks) and inhalation, and it is also absorbed through the skin. Its mechanism of action consists of (reversible) inhibition of cholinesterase enzymes (e.g., acetyl cholinesterase - AChE), leading to continued stimulation of muscarinic, nicotinic and CNS cholinergic synapses. As such, it is a neurotoxin, and exposure of humans and animals may cause clinical signs consisting of salivation, lacrimation, urination, defecation, other gastrointestinal symptoms, and emesis (SLUDGE syndrome); as well as tremors, stiffness, ataxia, dyspnea, seizures, coma, and death in severe cases. 3-OH-carbofuran is a metabolite of carbofuran in plants, insects, and mammals, and it is a known environmental transformation product of carbofuran and carbosulfan. Both the parent molecule and the metabolite are inhibitors of AChE.

Carbofuran products used to be registered in Canada for canola, mustard, sunflower, corn (sweet, field and silage), sugar beet, green pepper, potato, raspberry and strawberry crops; however, a re-evaluation by the Health Canada's Pest Management Regulatory Agency (PMRA) concluded that carbofuran products posed an unacceptable risk to humans and the environment, and did not meet Health Canada's standards for human health and environmental protection (RVD2010-16). As a consequence, carbofuran products could not be sold in Canada after 2010, and their application was prohibited after 2012. Despite the regulatory actions, these products have not been completely disposed of and animal poisoning, particularly in wildlife, is still identified (**Table 1**). *AHL*

Table 1. Historical cases of carbofuran toxicosis confirmed at the AHL (2009 - 2023).

Year (Province)	Species	Comments	Results
2009 (Ontario)	Canine	Two dogs acutely dead. Submitted bait (tissues) suspected to be laced with pesticides	Carbofuran detected in meat/muscle (bait)
Carbofuran revision: unavailable for sale after 2010; prohibited use after 2012			
2014 (British Columbia)	Bald eagle	Brain cholinesterase: 0.020 umol/g/min (17.21 +/- 3.18)	Carbofuran detected in liver and kidney
2016 (Saskatchewan)	Bald eagle	Eagle and a raven dead beside bag of grain. AChE 77% of normal	Carbofuran 19 ppm in crop content
2020 (Manitoba)	Fox and squirrels	Reduced AChE activity. In addition, unconfirmed dog and hawks also poisoned. A confession was made that Furadan was used.	No additional testing pursued.
2021 (Manitoba)	Bald eagles	4 bald eagles, a coyote and a raven found dead	Carbofuran detected in liver and crop content
2023 (Manitoba)	Bald eagles American red fox	Suspect wildlife poisoning	Carbofuran and 3-OH-carbofuran detected in liver

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Crenosoma vulpis in dogs

Jacob Avula, Tim Pasma

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

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Crenosoma vulpis, commonly known as the fox lungworm, inhabits the respiratory system (bronchi/bronchioles) of various canids, including domestic dogs. In Canada, this infection is relatively common in Atlantic region. To date, AHL has recorded four cases in dogs.

Adult female worms are ovoviviparous. The eggs are coughed up and swallowed, and the larvae hatch in the feces. These first stage larvae in the feces are eaten by terrestrial snails and slugs (intermediate hosts) and develop to infective third stage. When the definitive host ingests these snails, the larvae migrate to lungs, settle in bronchi, and mature. The pre-patent period is approximately 3 weeks.

Canine crenosomosis is usually characterized by bronchitis with a chronic dry cough and retching. Heavy parasitic burdens may induce mucopurulent discharge in the airways and the cough becomes productive. This may sometimes lead to a misdiagnosis of the condition as allergic respiratory disease.

The Baermann method of larval recovery remains the gold standard for the diagnosis of the infections caused by C. *vulpis* and also other lungworm infections, by taking advantage of the positive hydro/thermo-tropism exhibited by the live first stage larvae. For accurate identification of larvae, a thorough morphological and morphometric analysis must be done. The larvae measure 240-310 um and have a bluntly conical head. The tail tapers noticeably to a point with a slight deflection just before the tip (**Fig.1**).

Although the Baermann examination is recommended for diagnosing crenosomosis and other lungworm infections, this method has inherent limitations: it is relatively time-consuming (i.e., 12-24 hrs). False negative results can occur due to prepatent infections. Larvae are shed intermittently, a pattern typical of metastrongyloids, thus warranting repeated examinations (e.g., sample collected for three consecutive days). The sample must preferably be collected fresh (per rectum), as environmentally collected fecal samples can very quickly become contaminated with free-living nematodes, complicating the diagnosis.

When pulmonary parasitism is suspected, the veterinary clinician may also consider ordering fecal floatation/sedimentation in addition to Baermannisation, as *Paragonimus* and *Eucoleus* may also be involved, and they discharge only eggs in feces. An adequate amount of sample (~10 grams) must be sent to cover both tests.

At the AHL Parasitology lab section, a time-series observation was made on *Crenosoma* L1 recovery using the Baermann apparatus at different time points after storing the positive sample (moderate to heavy infection) in the refrigerator. The sample was set up by Baermann on days 3, 8, 14, and 17 after collection, and larvae were successfully recovered. However, in cases involving light infections where very few larvae are shed, the L1 recovery pattern may differ. *AHL*

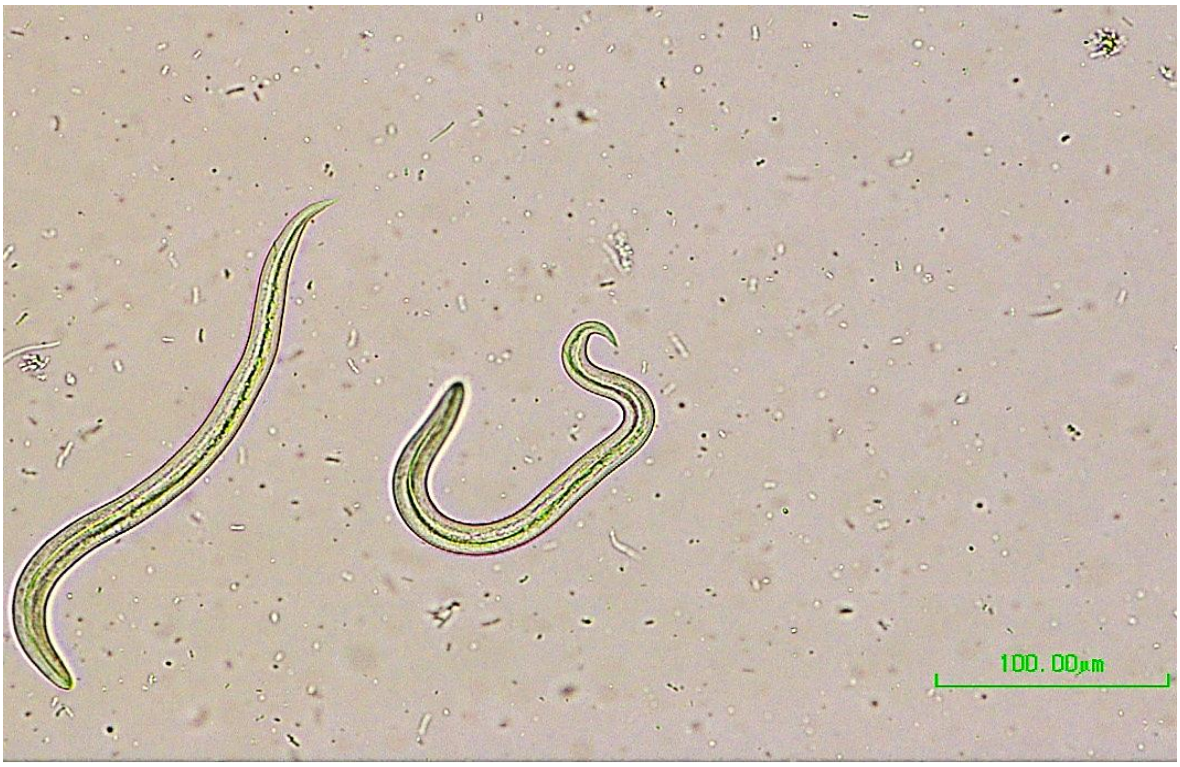

Figure 1. *Crenosoma vulpis* first stage larvae.

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Incidental *Trichinella*-type parasite in a canine eyelid biopsy for Meibomian gland adenoma excision

Rebecca Egan, César Rodríguez

Animal Health Laboratory, University of Guelph, Guelph, ON (Egan); Englehart Animal Hospital, Englehart ON (Rodríguez)

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A 14-year-old male neutered Shih Tzu dog presented to his veterinarian in Northern Ontario for assessment of a 1 cm diameter mass on the right lower eyelid. The mass had been present for 1.5 years and had been growing slowly until a recent rapid increase in size was noted. An excisional biopsy of the

eyelid mass was performed, along with a dental cleaning and extractions in the face of grade IV periodontal disease with concurrent neutrophilia. The eyelid specimen was sent to the Animal Health Laboratory for histopathology, and microscopic examination revealed a well-circumscribed 6 x 4 mm Meibomian gland adenoma expanding the skin at the mucocutaneous junction of the eyelid (**Fig. 1**). In the conjunctiva and dermis immediately surrounding the mass were lipid lakes and accumulations of macrophages that contained ample cytoplasm with many pieces of linear birefringent material (**Fig. 2**). This type of inflammation is characteristic of a chalazion which is a focus of lipogranulomatous inflammation incited by leakage of secretory material from a Meibomian gland adenoma. This inflammatory reaction likely contributed to the recent rapid growth noted clinically.

Microscopic examination of this specimen also revealed an unexpected finding. In the striated muscle of the eyelid adjacent to the mass, there was one enlarged myocyte with accumulation of fibrillar eosinophilic cytoplasm and multiple vesicular nuclei (nurse cell) surrounding a central intra-sarcoplasmic nematode larva typical of a *Trichinella* spp. parasite (**Fig. 3**). Diagnostic testing to confirm the species of parasite was not feasible, as only formalin-fixed paraffin-embedded tissue was available, and fresh muscle tissue is required for the standard muscle digestion test used for identification of this parasite.

Establishment of *Trichinella* spp. infection occurs following ingestion of meat harbouring parasitic cysts, with larvae eventually migrating throughout the body and forming intramuscular cysts in the host. These larval cysts may remain viable for years with no clinical signs of disease, therefore the majority of most infections in domestic and wild animals are likely to go undiagnosed. Interestingly, there is one recent European study where serologic assessment has been developed to utilize hunting dogs as a sentinel to monitor *Trichinella* spp. infections wildlife populations. In this dog, the precise source of the parasitic infection was unclear, but hunting of small rodents or consumption of a raw food diet were considered. The dog had been living in Northern Ontario in a rural setting close to farms and wooded areas for approximately 12 years, and while raw meat was not provided in the animal's diet at home and the dog had a primarily indoor life, scavenging of small rodents such as mice was a possibility. This dog had been acquired by the owners later in his life as a rescue, so the dog's lifestyle and exposure to wildlife prior to this period are unknown. *AHL*

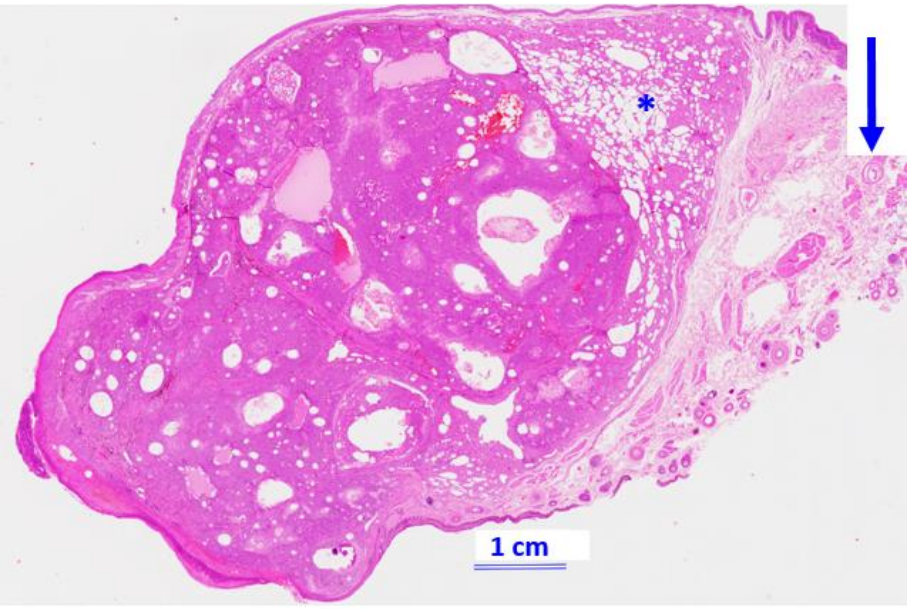

Figure 1. Microscopic section of eyelid (H&E, 1x) capturing an expansile Meibomian gland adenoma at the mucocutaneous junction accompanied by adjacent lipogranulomatous inflammation known as chalazion (*), and nearby striated muscle where there is a parasitic structure within a myocyte (arrow).

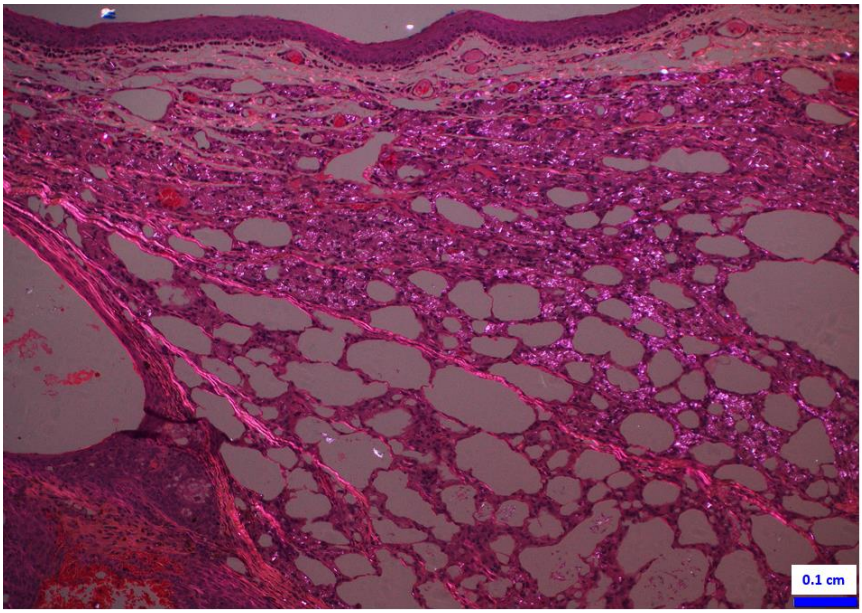

Figure 2. Microscopic section of eyelid (H&E, 10x, polarized light) capturing the chalazion, characterized by lipogranulomatous inflammation with linear birefringent material within the cytoplasm of macrophages (*).

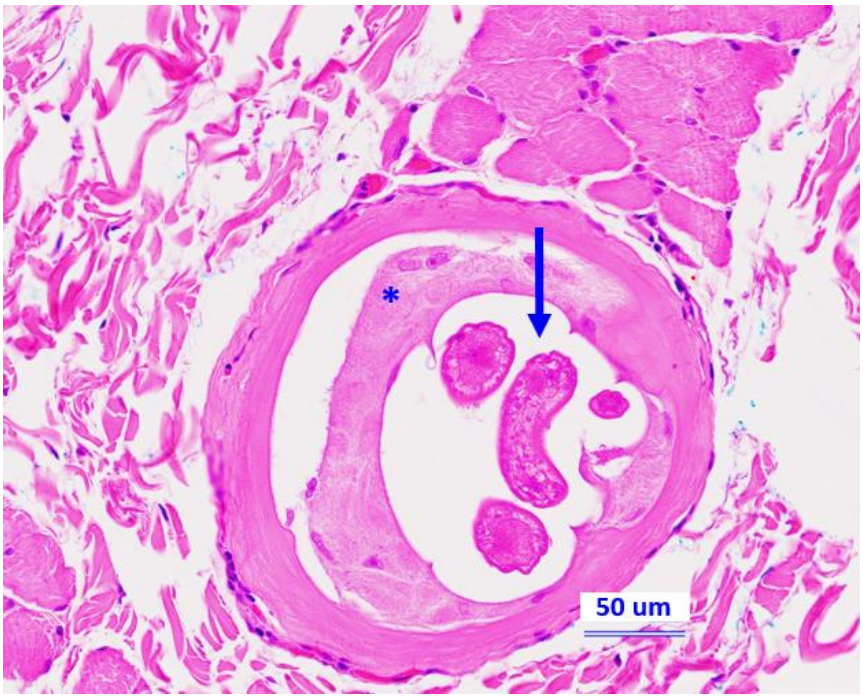

Figure 3. Microscopic section of eyelid (H&E, 20x) capturing a nematode larva (arrow) in a myocyte with nurse cell formation (*) compatible with *Trichinella* spp.

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