Laboratory Services Division
Animal Health Laboratory

AHL Newsletter

AHL Newsletter, Volume 29, Number 1 **March 2025**

In this issue:

AHL Newsletter
December, 2024 - Volume 29, Number 1 ISSN 1481-7179
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The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.
Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the editor.*

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Update from the Director

The view from the Director's office

Just when we were breathing a (tentative) sigh of relief that control efforts to prevent highly pathogenic avian influenza (HPAI) from infecting Canadian dairy cattle appear to be highly successful, along comes notification of 2 separate spillover mutation events involving the D1.1 genotype of H5N1 clade 2.3.4.4b that have infected dairy cattle in Arizona and Nevada. We can no longer be complacent that strict border biosecurity will protect our cattle, as genotype D1.1 is circulating in wild birds throughout Canada and is the dominant strain affecting poultry flocks in this country. Therefore, we need to remain vigilant and expect that similar homegrown favourable mutations will occur and affect our cattle. Kudos to all the bovine practitioners who have submitted samples for HPAI testing from sick cattle in their practices. Despite the justifiable fear and anxiety that a positive diagnosis would engender for clients, it behooves all of us to continue to test sick cattle, birds, cats and peridomestic mammals for HPAI, so that outbreaks can be contained quickly and the risk to humans mitigated.

It has been a challenging winter in Ontario with 8 positive poultry flocks diagnosed with HPAI since mid-December. AHL found itself in the unfamiliar circumstance of being located in one of the infected primary control zones. This requires all of our clients who submit diagnostic samples from a poultry facility to apply for a CFIA general movement permit in order to comply with movement restrictions. Most control zones are in place for approximately 3 months, and we will certainly inform you as soon as movement permits are no longer required. A huge thank you to all affected veterinarians and poultry industry clients for your patience during this time!

Maria Spinato, Director

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

AHL new tests developed in 2024

Helen Oliver

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AHL Newsletter 2025;29(1):3.

TEST NAME METHOD	CODE	SPECIES
Avian metapneumovirus type A, B, C (AMPV A, B, C) - PCR	ampvabc	avian, chicken, turkey
Avibacterium paragallinarum - PCR	avipcr	avian, chicken, turkey
Bacterial culture, aerobic and anaerobic, minimal inhibitory, companion/other	anculnm	avian, canine, equine, feline, other
Bacterial culture, aerobic, minimal inhibitory concentration, companion/other	cultnm	avian, canine, equine, feline, other
Bacterial culture, fecal, porcine	cultsfe	porcine
Canine C-reactive protein - photometric	crp	canine
Chicken anemia virus genotyping	cavseq	avian, chicken
Chicken rotavirus A/chicken rotavirus D/chicken parvovirus - PCR	chrppcr	avian, ovine, chicken
Epizootic haemorrhagic disease virus - antibody ELISA	ehdveli	bovine, caprine, ovine
Equine adenovirus/Equid herpesvirus 1&4/Equid herpesvirus 2&5 PCR	eadvehv	equine
Equine respiratory PCR panel	eresp	equine
Fish *Flavobacterium columnare* (Columnaris Disease) – PCR	fcolpcr	other
Fish *Lactococcus* culture, farmed fish	fishlac	other
Fowl adenovirus 11 virus neutralization test	fad11vn	avian, chicken
Fowl adenovirus 8a virus neutralization test	fad8avn	avian, chicken
Fowl adenovirus 8b virus neutralization test	fad8bvn	avian, chicken
Hatchery, environmental culture PCR	hsfepcr	avian, chicken, turkey
Mycoplasma hyopneumoniae - ID Screen antibody ELISA	mhyoeli	porcine
Ornithobacterium rhinotracheale - PCR	ortpcr	avian, chicken, turkey
Porcine, bacteriology enteric panel 1	pentpa1	porcine
Salmonella typing - CTS	bssero	avian, bovine, canine, caprine, chicken, equine, feline, ovine, porcine, turkey, other
Selective media for Lactococcus species isolation in fish	lacagar	other

Increase in USDA NVSL testing fees

Tim Pasma, Jennifer Zoethout

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AHL Newsletter 2025;29(1):4.

The United States Department of Agriculture recently announced increases in fees for testing conducted at the National Veterinary Services Laboratories in Ames, Iowa. As these fees had not changed since 2012, the resulting increase this year is substantial. The prices of the following tests will be affected:

Botulism MIT
Brucella canis ME tube agglutination test
Brucella ovis antibody ELISA
Dourine CF
Epizootic hemorrhagic disease AGID
Equine encephalitis virus – Eastern IgM ELISA
Equine encephalitis virus – Eastern and Western PRNT
Glanders
Malignant catarrhal fever VN
Piroplasmosis – *Babesia equi* and *Babesia caballi* cELISA
West Nile virus IgM ELISA
West Nile virus PRNT

Prices for these tests are available with a client account on our website at:
https://www.uoguelph.ca/ahl/tests

Complex histopathology cases and associated fees

Emily Brouwer

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AHL Newsletter 2025;29(1):4.

The fee structure for histopathology cases at the Animal Health Laboratory is divided into food animal cases and companion animal/other species cases.

Food animal histopathology is a flat fee, but there are additional fees for cases where there are excessive tissues or extra slides prepared. For cases where there are tissues requiring pathologist examination, trimming, or sampling, an additional fee for pathologist time will apply. Food animal cases where pathologist time is routinely applied includes, but is not limited to, subsampling tissues such as legs or plucks. Cases where tissues need additional processing or demineralization, such as bone or hoof, will incur additional charges for decalcification or hoof softening, respectively.

Non-food animal histopathology cases are separated into categories of histcm1, histcm2, or histcm3 based on the number of tissues provided and/or the overall size of the tissues. Larger tissues or complex

cases such as amputations, intestinal resections, splenectomies, large tumor resections, mammary chains, brains, etc. are automatically included in the histcm3 level. These criteria are listed on the back of the histology submission form, with examples. Similar to their food animal counterparts, those cases where pathologists are required to examine and sample the tissues are subject to additional fees for pathologist's time. Tissues requiring additional processing, such as demineralization or nail softening will also incur additional fees. Tumor margin assessment is available upon request for specimens larger than 2 cm, and this fee applies per tumor submitted.

If there is ever any confusion surrounding which tests to choose, or how to appropriately quote histopathology fees for your client, an avian or mammalian pathologist is available to help you. Please do not hesitate to contact us.

OAHN Update – March 2025

Mike Deane, Tanya Rossi

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The Ontario Animal Health Network (OAHN) has had a busy start to the winter, creating new projects, resources and reports for veterinarians, animal owners, and industry. The OAHN Companion Animal Network has put together a new info sheet on parvovirus, the Wildlife Network has completed a project on botulism test development, and the Aquatics Network is hosting an Indigenous Fish Health Day.

Need-2-Know: Parvovirus for dog owners

The OAHN Companion Animal Network created a new factsheet for dog owners on parvovirus, in response to an increase in cases in Ontario. It is a high-level factsheet for dog owners and community members in high-risk areas and scenarios to help raise awareness about canine parvovirus, how serious it can be, and basic prevention and control measures, with emphasis on the importance of vaccination. **https://www.oahn.ca/resources/parvovirus-dog-owners/**

OAHN Wildlife Project: Botulism Test Development

Drs. Claire Jardine (OVC), Alex Reid (OMAFA), and Durda Slavic led this OAHN-funded project to develop a PCR-based detection assay for the 6 *C. botulinum* toxin types A, B, C, D, E, and F. The development of this test by the AHL has filled an important gap, providing a PCR-based detection assay for botulism toxin genes that is now commercially available for veterinarians across Canada. Learn more here: **https://www.oahn.ca/resources/oahn-wildlife-project-botulism-test-development/**

Indigenous Fish Health Day

The Ontario Animal Health Network for Aquatic animals will present information and answer questions in a non-regulatory, open discussion format. OAHN is dedicated to information dissemination and knowledge transfer.

Non-Ontario residents are welcome, but we will be focused on freshwater finfish. Read more and register here: **https://www.oahn.ca/resources/indigenous-fish-health-day/**

New Reports

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

Companion Animal Network - **https://www.oahn.ca/reports/veterinary-need-2-know-n2k-update-sep-dec-2024/**

- OAHN winter survey and lab data: Key results
- H5N1 avian influenza on the rise: More cows, more cats, raw milk / meat risks
- Rabies update
- *Hepatozoon canis* in a dog: Eastern ON
- FIP drugs update
- GI antimicrobial use guidelines published
- Small flock sheep and goat course for vets
- RHD update webinar

Equine Network - https://www.oahn.ca/reports/equine-veterinary-report-q3-2024/

- BITS 'N SNIPS (or "things we talked about on the network call")
- Network member reports
- Syndromic and lab surveillance dashboard
- Equine research
- ResearchONequine

Small Ruminant Network -https://www.oahn.ca/reports/small-ruminant-veterinary-2024-apr-sep-review/

- Q2 & Q3 2024 Animal Health Laboratory case data
- Provincial slaughter & condemnation data
- HPAI in U.S. livestock update
- Small flock sheep and goat course for veterinarians & veterinary professionals

Swine Network - https://www.oahn.ca/reports/swine-veterinary-report-q3-2024/

- H5N1 Highly Pathogenic Avian Influenza (HPAI) detected in a smallholder swine farm in Oregon U.S.A.
- Update on H5N1 Highly Pathogenic Avian Influenza In U.S.A dairy cattle and in U.S. and Canadian poultry flocks
- Influenza A (IAV) - H3N2 clade 2010.1 detected for the first time in Quebec swine
- Porcine epidemic diarrhea virus (PEDV)/ Porcine deltacoronavirus (PDCoV)
- *Salmonella*
- OAHN veterinary clinical impression survey veterinary comments
- Laboratory diagnostic reports
- Ontario slaughter statistics
- CanSpotASF surveillance update
- OAHN project update: Porcine hemagglutinating and encephalomyelitis virus (PHEV)
- International disease topics of interest summary

Poultry Network - https://www.oahn.ca/reports/oahn-poultry-expert-network-quarterly-veterinary-report-q4-2024/

- Poultry veterinary survey highlights – Q4 2024
- Research

Staff highlights

AHL is pleased to announce that Marco Leung, BSc, MLS is the new Technical Supervisor of the Clinical Pathology laboratory. He has a background in pharmaceutical research, laboratory management, and regulatory compliance, with extensive experience in team leadership, operational optimization, and process improvement, ensuring efficiency and accuracy in high-stakes environments. Marco is a Registered Medical Laboratory Technologist in Hong Kong and holds a Bachelor of Science in Medical Laboratory Science. Welcome Marco!

Selected zoonotic pathogens and diseases in Ontario identified at the AHL in 2024

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AHL Newsletter 2025;29(1):9.

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

Case numbers for most zoonotic pathogens isolated or identified by the AHL in 2024 are relatively unchanged from the previous year, however some changes were identified. Avian West Nile virus positives have increased again this year, rising from 11 in 2021, 26 in 2022, 50 in 2023 to 76 in 2024. Case counts of equine West Nile virus have also risen from 5 in 2023 to 9 in 2024. 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].

After seeing an almost 4-fold increase in isolations of Salmonella enterica in chickens in 2023, case counts have returned to 2022 levels.

The percentage of animals identified as positive for leptospirosis was roughly unchanged in 2024 in cattle, equids, and swine, and the total number of submissions tested were similar as in 2023. However, submissions in canines increased by approximately 50% in 2024 with a moderate increase in percent positivity. 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.

Table 1. Number of cases* for selected zoonotic pathogens isolated and/or identified at the AHL in 2024.

AGENT	Bovine	Canine	Caprine	Chicken	Equine	Feline	Ovine	Swine	Turkey	Other**	2024	2023
Ascarids (incl *T. canis, T. cati, T. leonina, Baylisascaris* sp.)		5		55	7	2		20	1	10	**100**	93
Blastomyces dermatitidis		2				1					**3**	3
Bordetella bronchiseptica	6	1			7	1		30		6	**51**	49
Borrelia burgdorferi (Lyme disease), serology		29			18	2					**49**	37

Brucella sp. *(*non-*abortus)*											**0**	0
Campylobacter coli/ jejuni/ fetus subsp. *fetus*	5	1				2	8			1	**17**	7
Chlamydia sp.			11				10			3	**24**	16
Clostridium difficile					1			1		1	**3**	3
Coxiella burnetii (Q fever)	12		14				14			1	**41**	45
Cryptococcus sp.		1				2					**3**	0
Cryptosporidium sp.	160	1	14				2			4	**181**	140
Eastern equine encephalitis virus					13					2	**15**	10
Echinococcus multilocularis		22				1				9	**32**	15
Giardia sp.	11	10									**21**	45
Influenza A					2			178	23	81	**284**	382
Influenza A H5									7	69	**76**	138
Influenza A H7										5	**5**	2
Listeria monocytogenes	4		9		4		6			1	**24**	19
Methicillin-resistant *Staph aureus* (MRSA)	1	1			1					1	**4**	3
Methicillin-resistant *S. pseudintermedius* (MRSP)		67			1	4				5	**77**	97
Rabies virus											**0**	0
Salmonella enterica		1		15	1					10	**27**	81
Streptococcus suis	2			5				87		3	**97**	135
Streptococcus equisimilis	1	1			11			47		2	**62**	57
Streptococcus zooepidemicus	5				170	1					**176**	187
Toxoplasma sp.			1			2	4			1	**8**	13
Verotoxigenic *E.coli* (VTEC)	4										**4**	2
West Nile virus					9					76	**85**	50

Yersinia enterocolitica											**0**	2
Total	211	142	49	75	245	18	44	363	31	291	**1469**	1631

*Cases may include research samples
**Other species include wild avian species, and other domesticated and wild species

Table 2. *Leptospira* spp. seropositive, IHC-positive, or PCR-positive cases identified at the AHL in 2024.

***Leptospira* spp. serovar**	**Bovine**	**Canine**	**Equine**	**Swine**	**Other**
L. autumnalis	14	131	23	0	2
L. bratislava	11	32	23	1	2
L. canicola	17	85	13	0	2
L. grippotyphosa	6	62	3	0	2
L. hardjo	25	35	14	1	2
L. icterohaemorrhagiae	21	115	23	1	2
L. pomona	20	98	18	1	3
IHC or PCR-positive	0	1	0	0	0
Positive/tested cases	**33/151**	**156/237**	**31/51**	**1/16**	**3/9**
% pos	**21.8%**	**65.8%**	**60.8%**	**6.2%**	**33.3%**
% pos, 2024/2023	22/28%	66/51%	61/67%	6/7%	33/8%

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RUMINANTS

Bovine cutaneous melanocytic tumours

Amanda Mansz

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AHL Newsletter 2025;29(1):12.

Following the identification of a large, soft to gelatinous, diffusely black, cutaneous/subcutaneous, freely mobile mandibular mass in a mature black Angus bovid at slaughter, the mass was submitted to the AHL for histologic evaluation. On gross examination, the mass measured 14.0 cm x 11.0 cm x 7.0 cm, was covered by black-haired skin, and extended from the dermis to the deep subcutaneous adipose tissue (**Figs. 1a, 1b**). On histologic examination, the neoplastic mass was comprised of heavily melanized, plump round to spindle-shaped cells arranged in sheets, nests and packets, with some streams and whorls. Fewer than 1 mitotic figure was observed per 2.37mm2 microscopic field, and lymphovascular invasion was not seen. The benign features of the submitted mass, typified by the absence of lymph node metastasis, minimal cellular pleomorphism, and rare mitoses, warranted a diagnosis of cutaneous melanocytoma.

A retrospective publication on 10 melanocytic tumours in young cattle describes bovine melanocytic tumours as uncommon, but not rare, with an age between 2 months to 2 years at diagnosis, and Angus/Angus crossbred cattle as being over-represented. A distribution over the trunk was most often seen, but one was located on the jaw. In those animals available for post-surgical removal follow-up, no tumours had recurred.

In a search of the AHL database for melanocytic tumours in Bos spp. since January 2015, a total of 2 malignant melanomas, 5 benign melanocytomas, and 3 “melanocytic tumours” were diagnosed. Tumours had a generalized anatomical distribution with locations described on the maxilla, fetlock, flank, abdomen, tail, elbow and carpus in young bovids (less than 2 years of age). Criteria of malignancy for melanocytic tumours is not well-defined in cattle; however, the two diagnosed malignant melanomas did display lymph node metastasis. Scott’s Color Atlas of Farm Animal Dermatology suggests 80-90% of these melanocytic tumours are benign, but may grow to a large size (50cm) and become necrotic and ulcerated, thereby justifying early removal.

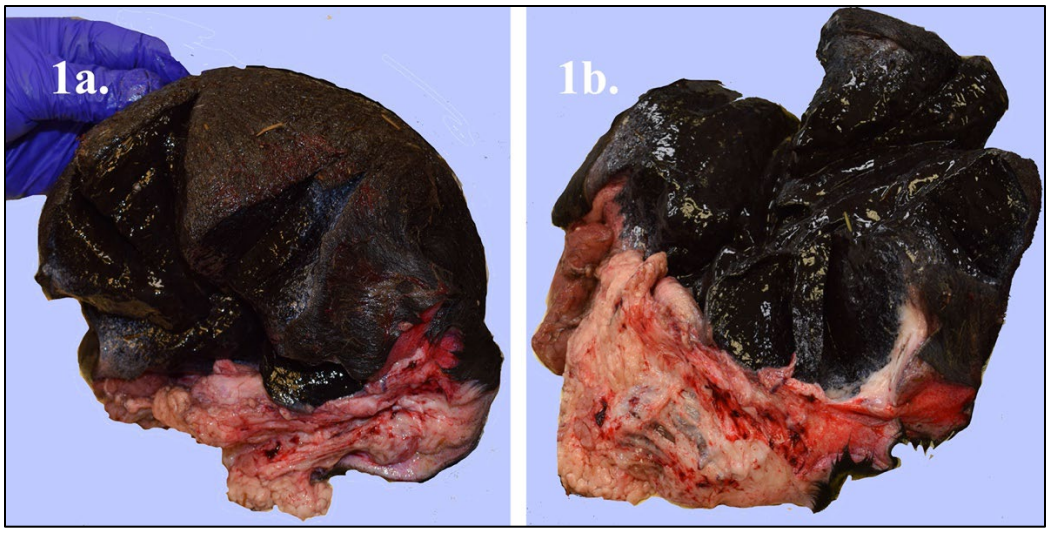

Figure 1. Cutaneous melanocytic tumour in a mature bovid**. 1a.** A large black melanocytic mass expanding the dermis extending to the deep subcutaneous adipose tissue. **1b.** A cut surface of the mass highlights the black pigmentation of the tumour.

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Perianal epithelioma in a goat and other caprine skin conditions

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AHL Newsletter 2025;29(1):13.

An adult Nubian goat was presented for a pedunculated mass just lateral to the anus that had reportedly arisen in the previous 36 hours. An excisional biopsy was performed, and the tissue was submitted for histological examination. An exophytic, pedunculated mass arose from the dermis at junction of haired and unhaired skin (**Fig. 1**). The mass was comprised of a central, fibrous tissue stromal core surrounded by multiple lobules of polygonal cells arranged in packets with rare, interspersed tubules or ducts that contained deeply eosinophilic fluid with scattered clear vacuoles. The epithelial cells had variably distinct cell borders and scant to abundant eosinophilic cytoplasm. Many of the cells exhibited basal cell differentiation, but some of the cells exhibited sebocyte-type differentiation (**Fig. 2A**). Approximately 60% of the mass was necrotic and included scattered neutrophils and abundant hemorrhage (**Fig. 2B**). The surface of the mass was ulcerated and coated with a serocellular crust containing scattered degenerate neutrophils and numerous colonies of basophilic coccoid bacteria (**Fig. 2C**). The mass was diagnosed as a suspect sebaceous epithelioma.

Caprine neoplasia is uncommon, with lymphoma, melanoma, fibropapilloma, squamous cell carcinoma, thymoma, and nasal carcinoma amongst the more common entities diagnosed. There are a few reports of sebaceous neoplasms in goats, usually sebaceous epitheliomas (two of which were also located in the perianal region), but prognostic information was not included. In general, sebaceous epitheliomas in cats and dogs are often cured by excision with adequate surgical margins. A few of these masses (in small animals) can metastasize to local lymph nodes - usually neoplasms excised from the head - but the prevalence of metastasis in goats is unknown.

Over the last 10 years at the AHL, 57 caprine cases have been submitted in which at least one skin condition was diagnosed (**Fig. 3**). The most common diagnosis was non-specific, often eosinophilic or neutrophilic dermatitis with hyperkeratosis (n=22); some of these cases were compounded by a superficial bacterial pyoderma. Differential diagnoses include ectoparasitism or ectoparasite hypersensitivity, bacterial dermatitis, *Malassezia* infection, zinc-responsive dermatopathy, or vitamin E/selenium deficiency. In rare cases with similar lesions, a specific cause was diagnosed: zinc deficiency in a goat with low serum zinc levels (0.44 ug/mL, ref: 0.65-2.7 ug/m); and *Malassezia* infection or mange in animals with intralesional organisms. However, follow-up testing and bloodwork were rarely available in a majority of cases. The second most common diagnosis (n = 12) was pediculosis (louse infestation), and these were all recorded in animals submitted for postmortem examination that were usually emaciated with concurrent debilitating disease. Bacterial dermatitis and necrotic/ulcerative skin lesions were diagnosed in 9 and 5 animals, respectively. Four cases of proliferative lesions were diagnosed in the past 10 years, including the previously described epithelioma along with a single case each of fibroma, squamous cell carcinoma in situ, and squamous papilloma. Abscesses, dermatophytosis, mange, cutaneous cyst, hematoma, *Malassezia* infection, hyperkeratosis, zinc deficiency dermatopathy and a laceration were diagnosed in three or fewer cases. Based on these described cases and the reviewed literature, neoplasia, abscess, cyst, granuloma, hamartoma, seroma, and hematoma are all reasonable differential diagnoses for cutaneous masses in goats. Cytology and/or histology may be required for definitive diagnosis.

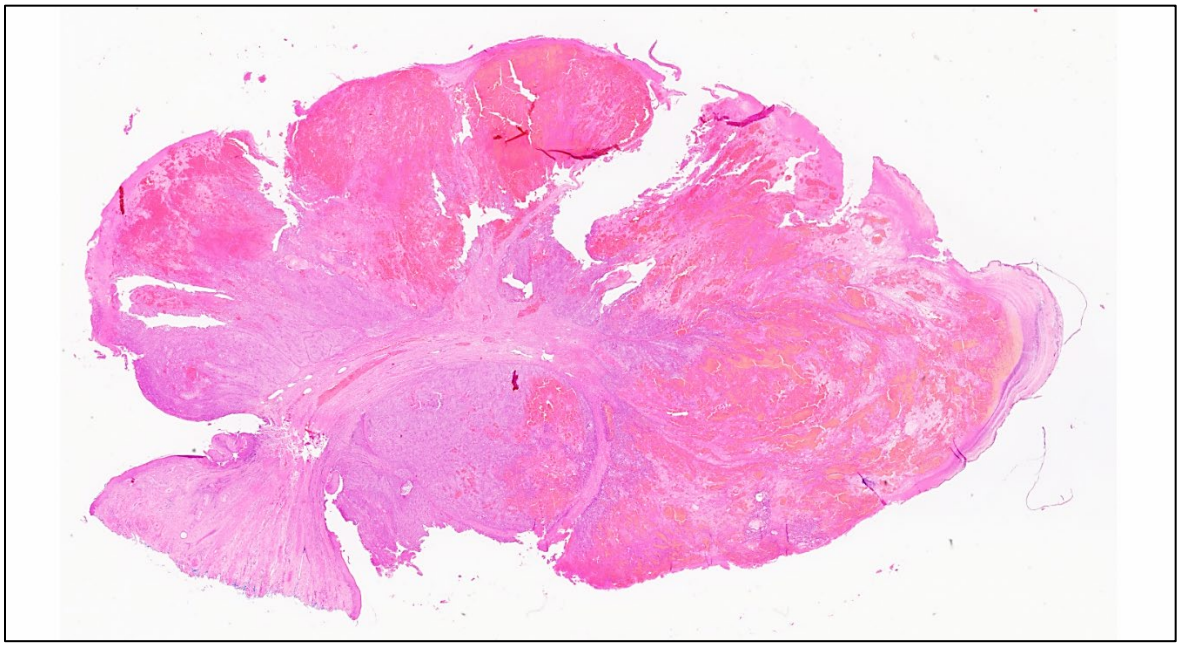

Figure 1. Perineum. An exophytic, pedunculated mass arises from the dermis at junction of haired and unhaired skin. Subgross, H&E stain.

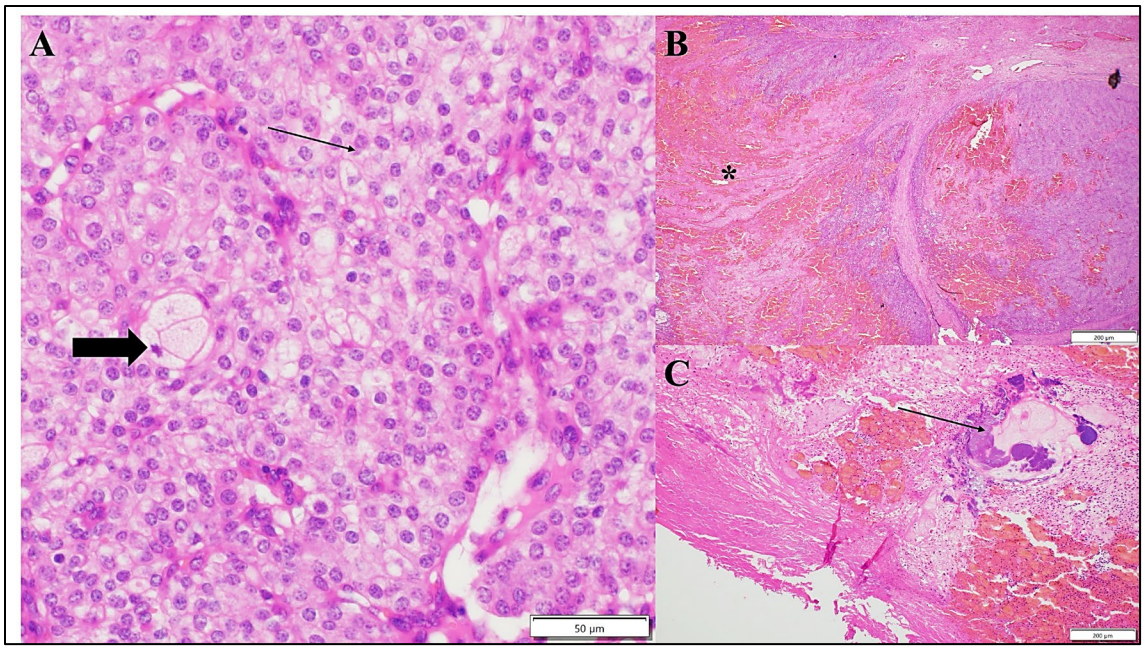

Figure 2. Dermis, perineum. H&E stain. **2A**. Most of the tumour cells exhibit basal cell differentiation (thin arrow), but some of the cells exhibit sebocyte-type differentiation (thick arrow). 40X. **2B**. Approximately 60% of the mass is necrotic and includes scattered neutrophils and abundant hemorrhage (asterisk). 10X. **2C**. The surface of the mass is ulcerated and coated with a serocellular crust with scattered degenerate neutrophils, and numerous colonies of basophilic coccoid bacteria (thin arrow). 10X.

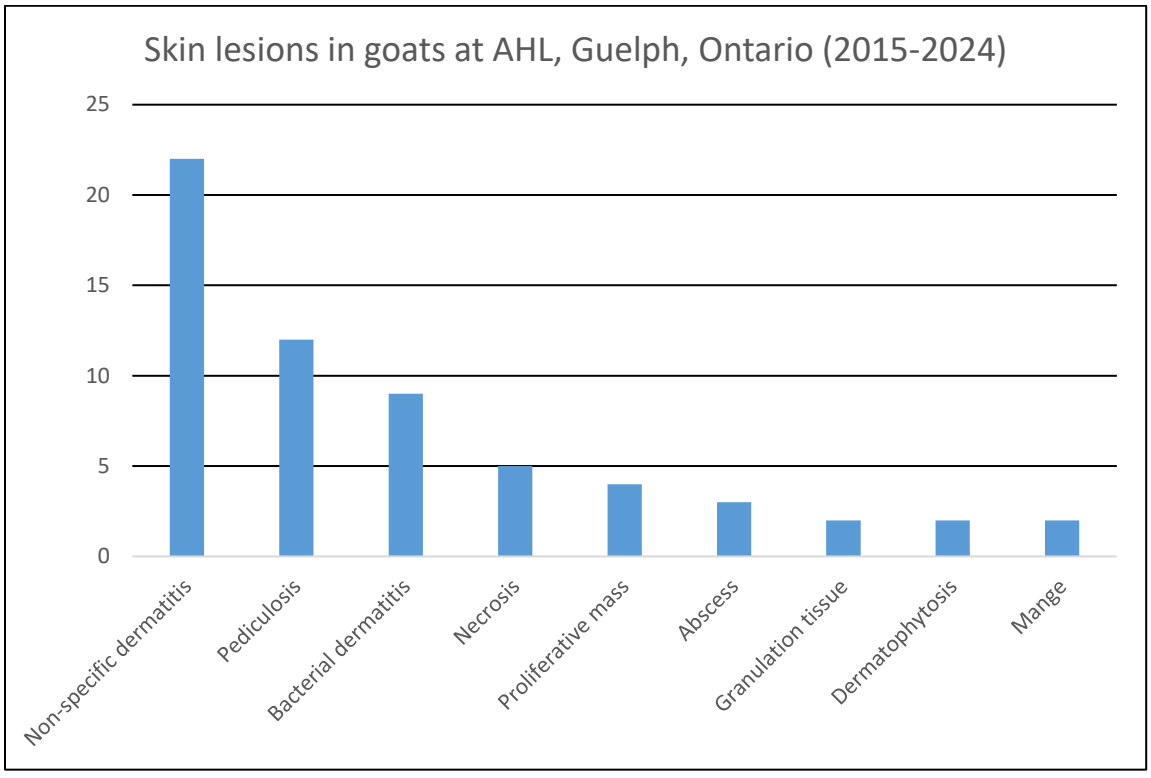

Figure 3. A total of 67 skin conditions were diagnosed in 57 caprine submissions from January 1, 2015 to December 31, 2024. Diagnoses with two or more recorded cases in this period are shown in the bar graph. Only a single case of each of the following conditions was recorded, so they were not included in the figure: cutaneous cyst, hematoma, *Malassezia* infection, hyperkeratosis, zinc deficiency dermatopathy and a laceration.

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Glaesserella parasuis PCR: A useful adjunct to bacterial culture

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AHL Newsletter 2025;29(1):17.

Isolation of *Glaesserella parasuis* from clinical samples is challenging using routine bacterial culture techniques. The inherent fragility of the organism and its fastidious growth requirements have a significant influence on successful culture, necessitating special media and conditions. Use of PCR techniques can increase the sensitivity of *G. parasuis* detection. For clinical cases (**Fig. 1**) with potential *G. parasuis* involvement, both *G .parasuis* PCR and routine culture for other bacteria are recommended to increase the likelihood of detecting bacterial pathogens.

G. parasuis PCR can be carried out on a range of samples including joint fluid, joint capsule, swabs from joints or other serous surfaces, lung, or tonsil. For swab samples, viral transport swabs (VTM swabs) should be used. **Avoid gel-based culture swabs for *G. parasuis* PCR testing**, as the gel has an inhibitory effect on PCR results. *Mycoplasma hyorhinis* PCR can be carried out on the same VTM swab samples for those cases with polyserositis or other lesions where this organism is also a potential etiologic agent. Concurrent routine bacterial culture is recommended using gel-based swabs or other samples from the same clinical sites, in order to investigate other pathogens not detected by organism-specific PCR tests.

Please contact the AHL with any questions regarding the use of *G. parasuis* PCR in diagnostic test plans for polyserositis and other bacterial diseases.

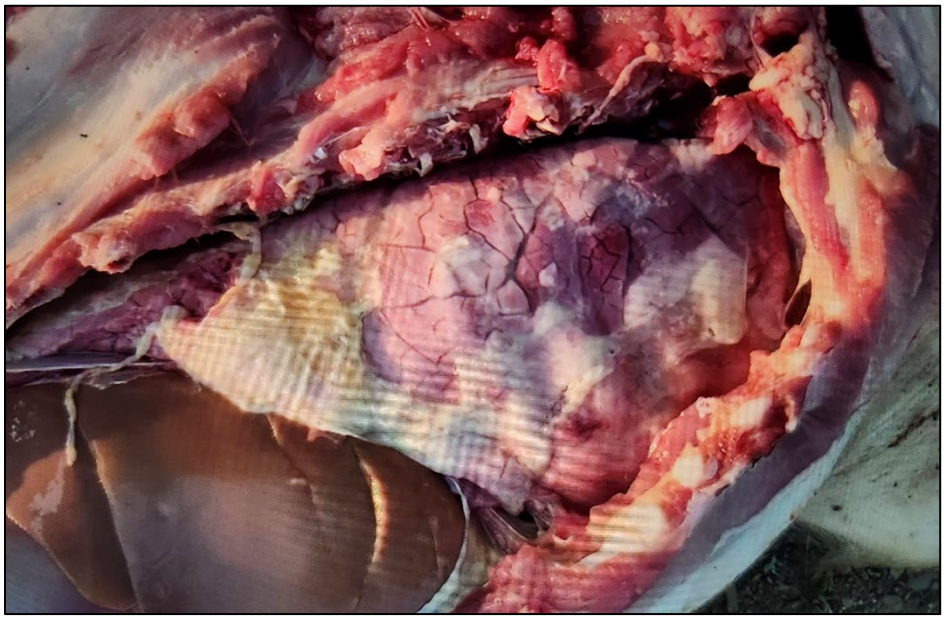

Fig. 1. Fibrinosuppurative pleuritis in a grower pig, typical of *Glaesserella parasuis* infection.

Genotyping of porcine reproductive and respiratory syndrome virus in Ontario

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AHL Newsletter 2025;29(1):18.

Genetic characterization of porcine reproductive and respiratory syndrome virus type 2 (PRRSV) is typically done through open reading frame 5 (ORF5) analysis. ORF5 encodes glycoprotein 5, a highly variable component of the PRRSV envelope that is critical in the infection process and in swine immune responses. Historically, strain classification was carried out by ORF5 restriction fragment length polymorphism (RFLP) analysis; later on, ORF5 phylogenetic analysis was introduced. Although RFLP analysis corresponds with phylogenetic analysis to some extent, phylogenetic analysis provides a more stringent discrimination of PRRSV strains.

Recently, a refinement of the PRRSV lineage-based classification was proposed, which subdivided PRRSV into 11 lineages (L1–L11) and 21 sub-lineages (L1A–L1F, L1H–L1J, L5A–L5B, L8A–L8E, and L9A–L9E), based on ORF5 sequences. Using these criteria, an analysis of 1,777 PRRSV ORF5 sequences obtained from Ontario samples between 2015 and 2024 recognized the presence of six lineages (L1, L2, L4, L5, L7, L8). The most frequently detected lineages were L1 and L5, with 1,423 (80.08%) and 325 (17.73%) samples identified, respectively. Detection of lineages L2, L4, L7, and L8 in Ontario was infrequent (**Table 1**). Lineages L3, L6, L9, L10, and L11 were not detected.

Sub-lineage classification assigned 1,250 sequences into 9 sub-lineages: L1B, L1C, L1D, L1E, L1F, L1H, L5A, L8A, and L8C (**Table 2**). However, 525 sequences in the L1 lineage could not be definitively classified using the current criteria. Ontario PRRSV ORF5 sequences with ambiguous or undetermined L1 sub-lineage designations were labeled with an “X” (e.g., L1X, L1X.1).

Some RFLP patterns were sub-lineage-specific; for example, all 2-5-2 sequences were in the L5A sub-lineage, and all 1-8-4 sequences were in the L1H sub-lineage. On the other hand, the RFLP pattern 1-1-1 was detected in four sub-lineages, and 1-1-2 was detected in five sub-lineages (**Table 3**).

Table 1. Lineage classification of PRRSV ORF5 sequences from Ontario, 2015-2024.

Lineage	Count	%
L1	1,423	80.08%
L2	6	0.34%
L4	1	0.06%
L5	315	17.73%
L7	2	0.11%
L8	30	1.69%
Total	1,777	100.00%

Table 2. Sub-lineage classification of PRRSV ORF5 sequences from Ontario, 2015-2024.

Sub-lineage	Count	%
L1B	40	2.25%
L1C	1	0.06%
L1D	29	1.63%
L1E	207	11.65%
L1F	2	0.11%
L1H	626	35.23%
L1X	60	3.38%
L1X1	465	26.17%
L5A	315	17.73%
L7	2	0.11%
L8A	27	1.52%
L8C	3	0.17%
Total	1,777	100.00%

Table 3. Top 10 most frequent RFLP patterns of PRRSV ORF5 sequences from Ontario, 2015-2024.

RFLP	L1B	L1E	L1F	L1H	L1X	L1X.1	L5A	L8A	Total
1-1-1		3	2			279		4	288
2-5-2							257		257
1-8-4				243					243
1-4-2				230	1				231
1-3-2		90		13	9				112
1-30-1						75			75
1-1-2	8	22				1	2	21	54
1-3-1		36			2	13			51
1-22-2		36							36
1-8-3				34					34

Reference

1.Yim-Im W, et al. Refining PRRSV-2 genetic classification based on global ORF5 sequences. Microbiol Spectr 2023;11(6):e02916-23.

AVIAN/FUR/EXOTIC

Update regarding avian influenza and CFIA permits for poultry samples

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AHL Newsletter 2025;29(1):20.

A reminder to all AHL clients that since early January 2025, the AHL has been located within a primary control zone instituted by the Canadian Food Inspection Agency (CFIA) in response to an outbreak of highly pathogenic avian influenza on a premises in the Guelph area.

As a result, a CFIA general control permit must accompany all poultry samples or carcasses that are submitted to the AHL for diagnostic testing. Submissions from pet birds and wildlife are exempt. Submissions to the AHL originating from other veterinary diagnostic laboratories are also exempt.

A CFIA general control permit can be accessed here: https://inspection.canada.ca/en/about-cfia/find-form/cfia-acia-5752. Detailed instructions for completing the permit are also posted on the AHL website: https://www.uoguelph.ca/ahl/news/2025/01/notice-shipping-avian-samples-ahl.

Once you receive your approved permit, please include it with each AHL submission – a printed copy may be included with your submission package, or it may be emailed to specroom@uoguelph.ca.

The requirement for a CFIA general control permit will remain in effect until the control zone is revoked by CFIA. AHL will notify clients as soon as the control zone is revoked and the movement permit is no longer required.

If you have any questions or concerns regarding permits, please contact CFIA at cfia.ontmovementlicandpermits-deplacementlicenceetpermis.acia@inspection.gc.ca

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Understanding the histopathology of reovirus lesions

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AHL Newsletter 2025;29(1):20.

It has been over a decade since reovirus emerged as a cause of lameness in Ontario chickens. Prior to 2012, bacterial and developmental causes of lameness were the primary diagnoses. Since then, various strains of reovirus have emerged in Ontario and have contributed to varying lameness severity. Reovirus has also emerged as a cause of lameness in turkeys.

When examining tissues from lameness cases, we prefer to have both heart and gastrocnemius tendon (**Fig. 1A**) to examine as some reovirus lesions can be more developed in one tissue versus the other (**Figs. 1B,1C, 1D**). We also prefer to have 3 to 5 sections of each tissue to examine since not every tissue may contain lesions.

When sampling lameness cases for ancillary testing, it is important that the PCR and histopathology samples be collected from the same birds so the results can be correlated. Reovirus PCR is routinely run in conjunction with histology of at least gastrocnemius tendon (**NOTE**: flexor tendon is also required in turkeys). The combination of a positive PCR test as well as lesions of lymphoplasmacytic tenosynovitis and epicarditis on histopathology confirms the presence of a reovirus infection. Occasionally, there are other combinations of results that can be confusing. Sometimes there will be no histologic lesions suggestive of reovirus infection, but the reovirus PCR will be positive. This may indicate the virus is just being introduced into these birds and lesions have not yet developed. In other situations, there may be obvious well-developed histologic lesions (**Figs. 1C, 1D**) that pathologists consider consistent with or highly suggestive of a reovirus infection, but the reovirus PCR is negative. In this case, the reovirus organism may no longer be present in the tissue, but there has been sufficient time for severe lesions to develop. Based on case experience and the current disease environment in Ontario, lymphoplasmacytic tenosynovitis in broiler chickens is primarily caused by reovirus; however, similar lesions could be caused by *Mycoplasma synoviae*. In other chicken commodity groups (i.e., broiler breeders, layers) and turkeys, other disease rule outs should still be considered.

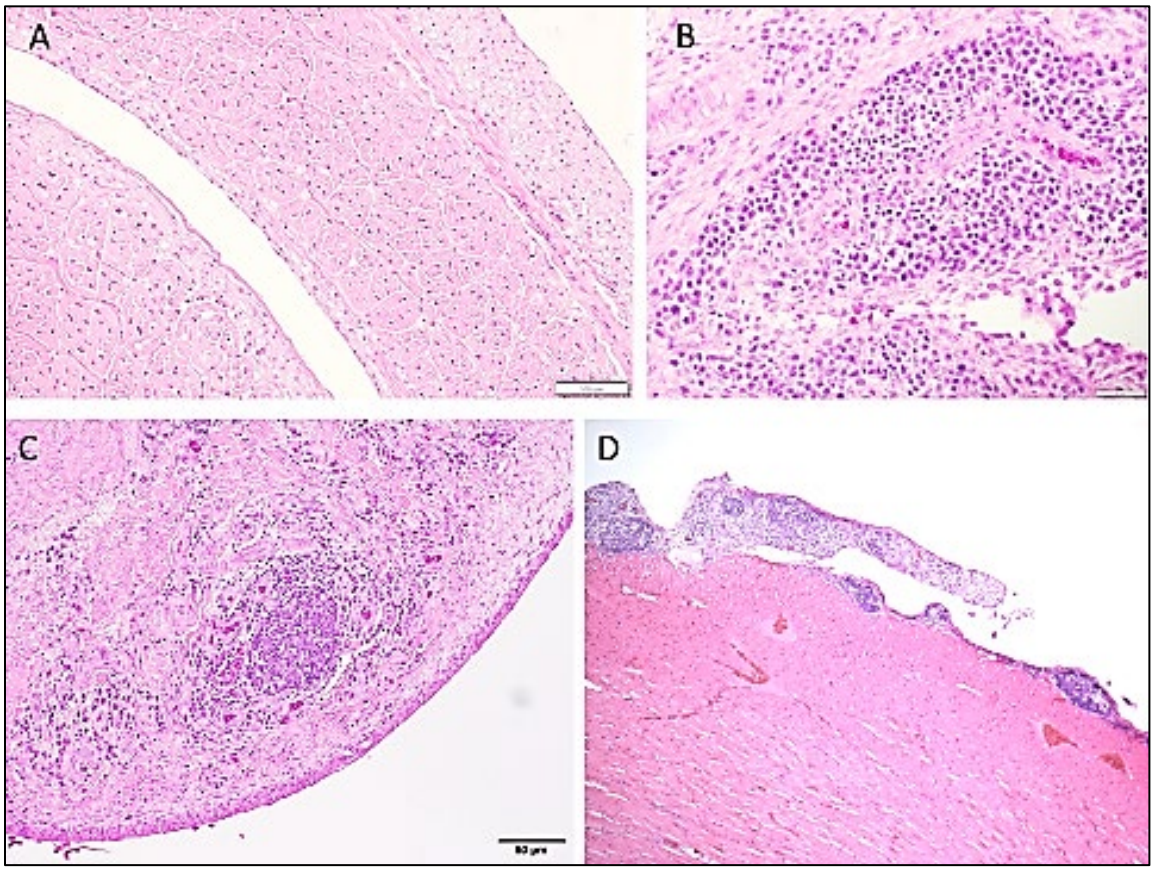

Figure 1. A. Normal tendon. **B.** Predominantly lymphocyte and plasma cell populations in the synoviae. 40X **C.** Development of a lymphoid nodule within the gastrocnemius tendon. 20X **D.** Heart. Multiple lymphoid nodules over the epicardium. H&E stain.

Pemphigus foliaceous in a cat: Cytologic - histologic correlation

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AHL Newsletter 2025;29(1):22.

A 6-year-old male neutered Ragdoll cat presented to the referring veterinarian with a 2- to 3-week history of progressive erythematous crusting skin lesions affecting the right ear, left axilla, left upper forelimb and front digits (**Fig. 1**). The cat was housed indoors, and there was no history or evidence of trauma. Clinical differential diagnoses included infectious agents, allergic reactions and immune-mediated disease. Initial treatment consisted of antibiotic injections (Covenia®), local antiseptics (chlorhexidine) and a topical anti-fungal/antibacterial/anti-inflammatory preparation (Otizole®).

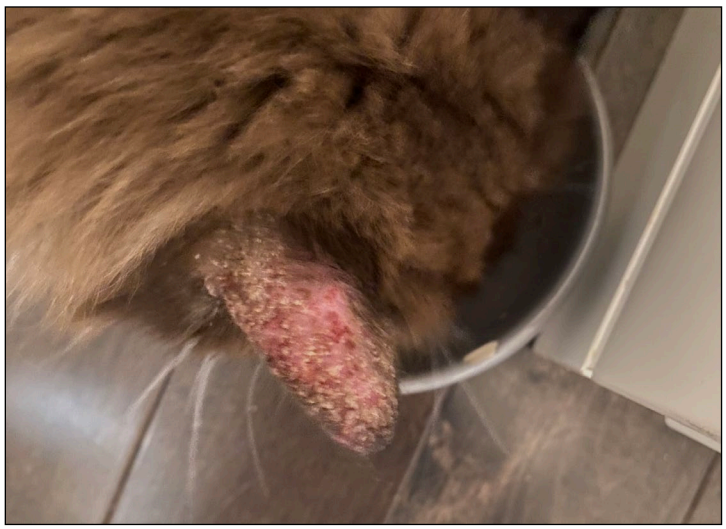

Figure 1. Ulcerative, crusting skin lesions. Right ear.

Several tests were performed to investigate the etiology of this process. PCR for fungal organisms, examination for ectoparasites (*Demodex* spp., *Sarcoptes* spp.) and culture for dermatophytes (ringworm) were all negative. *E. coli* was isolated from an ear swab. Impression smears from the cutaneous lesions on the right ear and paws were submitted to the Animal Health Laboratory (AHL) for cytological interpretation. These samples consisted of poorly preserved squamous epithelial cells intermixed with numerous neutrophils against a densely proteinaceous background with abundant debris (**Fig. 2A**). Intracellular bacteria or fungal organisms were not identified. The epithelial cells included a few individual hyperchromatic cells with discrete rounded cytoplasmic borders and small pyknotic nuclei. These were compatible with “acantholytic keratinocytes”, raising concerns for immune-mediated disease, specifically pemphigus foliaceous (**Fig. 2B**). Histopathology was recommended for confirmation.

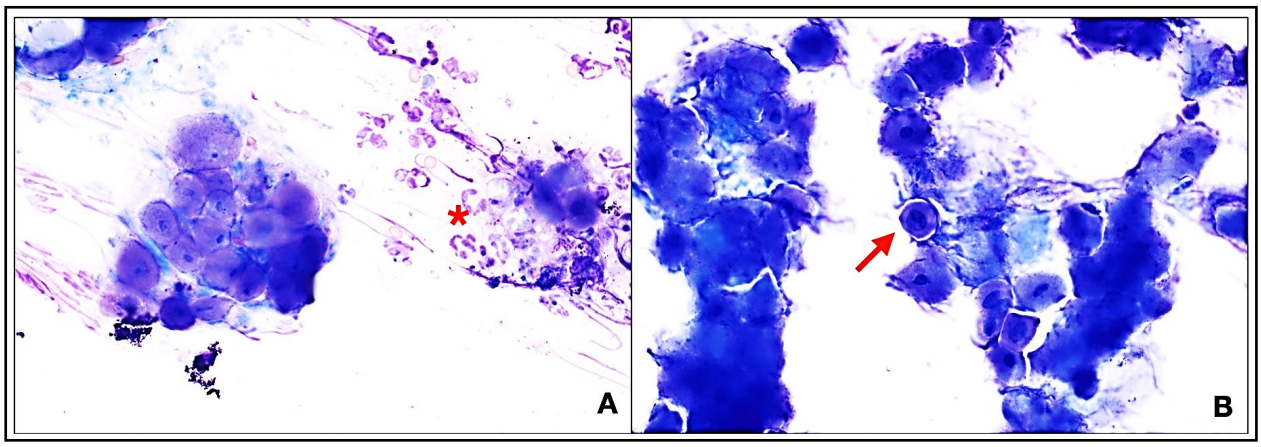

Figure 2. Impression smears of cutaneous lesions on front digits. 2A. A cluster of keratinocytes (left) and neutrophilic infiltrates (asterisk). 2B. Acantholytic keratinocytes (arrow) amongst poorly preserved squamous epithelial cells and debris. Wright's stain, 60x.

Multiple 6 mm cutaneous punch biopsies from the right pinna and left axilla were submitted to the AHL for histopathologic examination. Histologically, the epidermis was moderately hyperplastic, with multiple variably sized subcorneal and intraspinous pustules present. Within each pustule, there were several acantholytic keratinocytes and non-degenerate neutrophils (**Figs. 3A, 3B**). These histological findings confirmed the presumptive clinical and cytological diagnosis of pemphigus foliaceous.

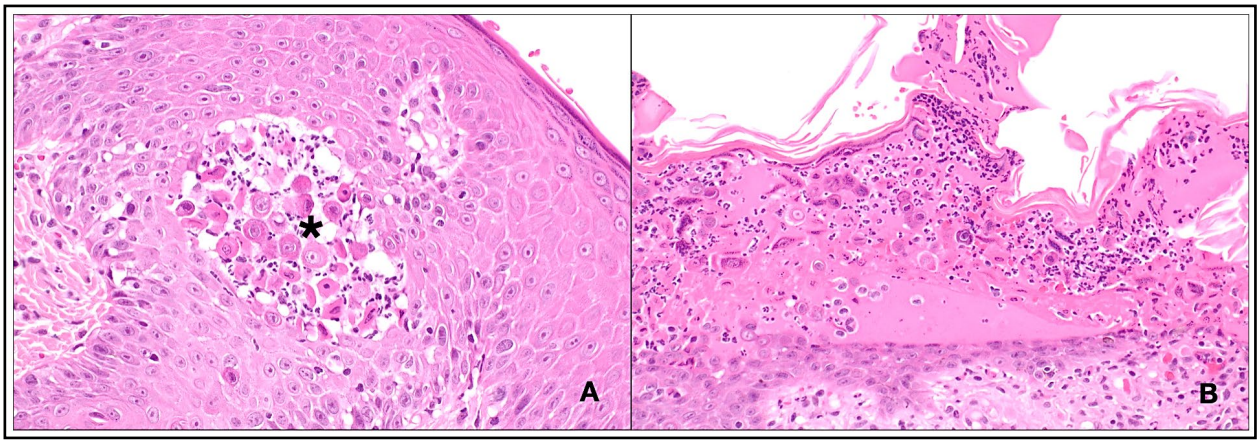

Figure 3. Histology of punch biopsies from cutaneous lesions. 3A. Intraepidermal pustule with acantholytic keratinocytes (asterisk) and neutrophils. H&E stain, 40X. 3B. Serous exudate with neutrophils, acantholytic keratinocytes and cellular debris. H&E stain, 20X.

Pemphigus complex is a cutaneous/mucocutaneous immune-mediated disorder characterized by blisters, pustules, erosions and ulcers of the skin, caused by decreased adhesion of epidermal keratinocytes (acantholysis). Pemphigus foliaceous specifically affects haired skin. It is associated with production of autoantibodies targeting desmoglein-1, a protein present in desmosomes, potentially disrupting integrity of adhesion molecules.

The characteristic distribution of cutaneous lesions in this patient (head and paws), and the cytological and histological evidence of acantholysis confirm the diagnosis of pemphigus foliaceous. Cytological examination of impression smears from **newly-formed** lesions can be an easy, non-invasive and inexpensive screening test, keeping in mind that samples may contain mostly debris and/or the lesions become secondarily infected, making the cytological interpretation challenging. Histology of multiple skin biopsies from acute lesions may provide the key architectural features of the lesions required for confirmation of this diagnosis.

Reference

1. Mauldin EA, Peters-Kennedy J. Integumentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, 6th ed. Maxie MG, ed. Elsevier, 2016;vol 3:600.

Get some skin in the game; Ways to improve diagnostic yield on skin biopsy submissions

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AHL Newsletter 2025;29(1):24.

This article is an update of a prior AHL Newsletter article (Dec. 2003;7(4):39) by DeLay et al: 'Show me some skin - obtaining useful information from skin biopsies'.
Dermatologic disease can be very frustrating for everyone involved, from patient and client to the treating veterinarian and pathologist. In many species, these can present with a variety of relatively non-specific clinical signs, including hair loss, pruritis and variable changes involving the skin surface such as crusting, scaling or reddening of the skin. Histology can play a helpful, if not critical, role in diagnosing the underlying cause. However, there are several important things to consider when taking a biopsy for diagnostic purposes.

Time of biopsy: *Sample a variety of lesions in varying degrees of progression.* Lesions can change over the course of the disease process; it helps to know how the disease progresses. Often as the barrier function is lost during the progression of the skin disease, the histological and gross appearance becomes complicated by a secondary superficial bacterial infection. Therefore, either an early disease process is obscured by secondary inflammation, or the results of that chronic inflammation (scarring, etc.) can mask the lesion, resulting in a non-specific diagnosis. Sampling from earlier in the disease course (prior to secondary infection), or sampling after an initial course of antimicrobial therapy is helpful as it reduces the secondary skin lesions. Primary skin lesions include macule/patch, papule/plaque, pustule, vesicle/bulla, wheal, nodule or cyst. Lesions that may be considered primary or secondary include alopecia, scale, crust, follicular casts, comedo and pigmentary abnormalities. Secondary lesions include epidermal collarette, scar, excoriation, erosion/ulcer, fissure, lichenification and callus. Sampling a variety of stages is key!

Treatment considerations: *Glucocorticoid treatment can change the appearance of the skin and thereby affect diagnosis.* While early antimicrobial therapy can allow for reduction of the secondary bacterial infection that complicates interpretation of the histological lesions, glucocorticoid treatment can greatly affect the appearance of the skin. Therefore, there should be a period of 4-6 weeks between the last

glucocorticoid treatment and skin biopsy to allow for the effects of the therapy to fade. The duration of treatment and date of last treatment should be included in the submission history

Dermatologic history: *Lesion distribution can allow for differentiation among specific disease processes.* Remember to include the owner's chief complaint, the animal's age, breed, sex and coat colour, and always include the lesion distribution in your clinical history. Dermatological diseases tend to have specific anatomic distributions, and the ability to correlate the histological findings with the gross location is very helpful in diagnosis. Depending on the disease, you may see preferential targeting of face, nasal planum, mucocutaneous junctions, dorsal back, etc. Providing this information in the clinical history allows the pathologist to correlate the histological findings with the known disease targets. It is also important to provide information regarding any previous treatments and the results of these therapies, as these can impact the histological features. Photographs can always be included with case submissions, and are always welcomed.

Multiple biopsies (Fig. 1): *A minimum of 6 biopsies is recommended, especially for most equine and food animal dermatological cases*. In many dermatological diseases, the histological appearance can vary depending on when the sample is taken during the development of the lesion (the appearance can also vary depending on the area of the body affected), or if there are secondary skin changes that occur in more chronic disease processes (i.e., secondary bacterial infections). Therefore, multiple biopsies from the various affected sites are recommended, as this gives the pathologist a better picture of the spectrum of lesions observed. This is especially important in large animals with a greater skin surface area (i.e. equine and other farm animals).

Talk to us: *If the report does not match the clinical appearance or there is an inappropriate/inadequate response to therapy, please reach out to us.* While histology can provide a great deal of information about dermatological diseases, it is the summation of the diagnostic work up that helps provide a diagnosis. The most useful diagnoses can be made when the microscopic lesions are interpreted in association with the clinical picture. Including your list of differential diagnoses is a helpful way of sharing with us what you are seeing clinically, and at the very least, will allow us to rule in/out your disease of concern.

Resubmissions are sometimes necessary: *Sometimes the initial biopsy does not capture the full picture due to many of the considerations described above.* If the histologic results do not appear to match the clinical picture, a resubmission can sometimes provide more diagnostic information.

While skin diseases can be challenging to work up, a few small changes in sample selection and submission can go a long way toward providing a diagnosis. Our hope is that these recommendations and the quick tips listed below (**Table 1**) will help you to maximize the diagnostic return on your future skin biopsy submissions.

Table 1. Quick tips for maximizing the diagnostic utility of skin biopsies for histopathologic evaluation

1.	A **6 mm** biopsy punch provides an adequate specimen; **4 mm** should be reserved for difficult sites, cats or small dogs.
2.	Leading edge lesions are good, but **do not include too much normal skin** because depending on the orientation/rotation the lesion could be missed at trimming.
3.	Try to **center your lesion** (papules/pustules/vesicles) in the biopsy
4.	**Compare** your report to your clinical lesions (did the report not describe the vesicle you submitted? If not, we may need to re-trim).
5.	**Immediately submerge** your biopsy in the appropriate fixative (formalin); Don't wait until all the samples are taken to place them in formalin.
6.	**Consider using a scalpel** for 1. larger lesions, 2. vesicles/bullae/pustules (shearing action of the punch may damage the lesion), and 3. suspected disease of the subcutaneous fat (as a punch may not provide a sample that includes that deeper tissue).

7.	**Avoid shaving and scrubbing** (clip hairs only if necessary) to keep the keratin layers intact. The skin surface should be left untouched or gently dabbed with alcohol.
8.	If the disease is potentially infectious, remember **Lidocaine** can **inhibit growth** of gram positive and gram negative bacteria, mycobacteria and fungi. As an alternative, consider a ring block or general anesthesia in these cases.
9.	**Avoid forceps** and consider using tiny mosquito hemostats, Adson thumb forceps or a syringe needle to handle the sample. Larger forceps can result in significant crush artefact, complicating interpretation.

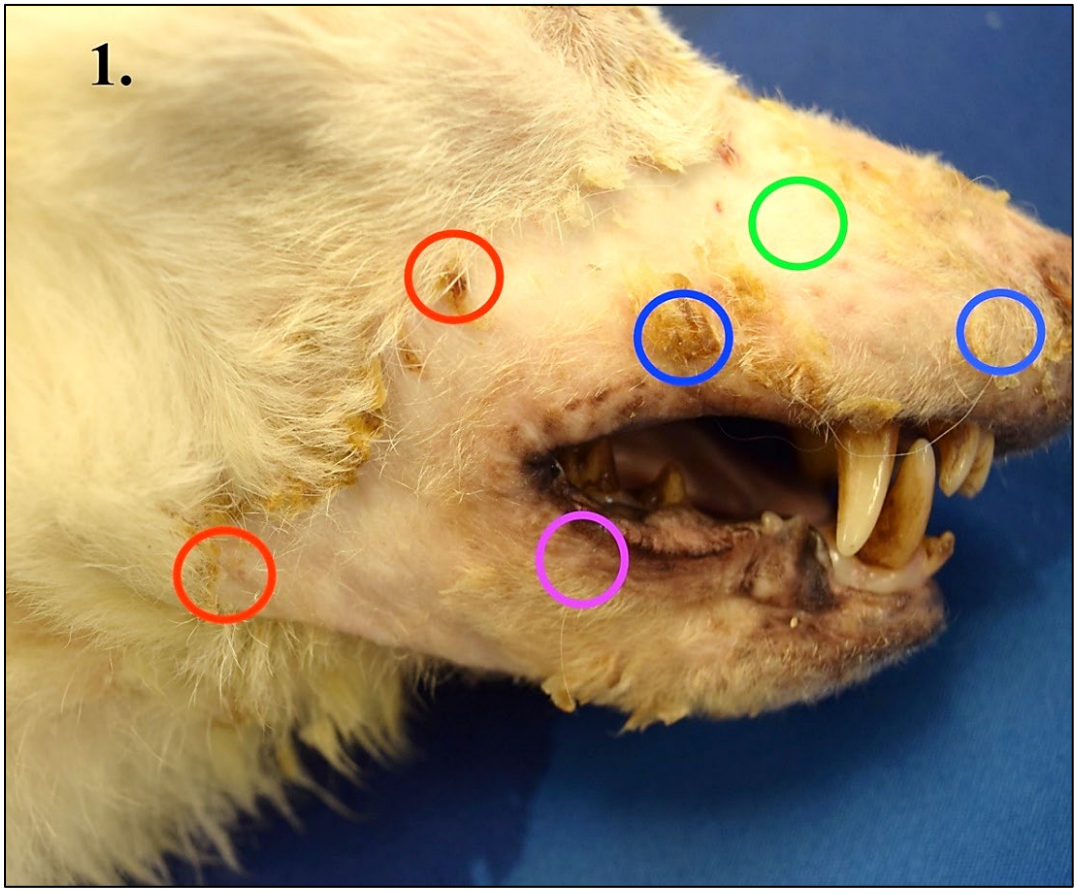

Figure 1. Appropriate skin biopsy selection. A minimum of 6 biopsies from variable sites are recommended. The red circle represents a sample from the leading edge of the lesion, progressing from normal-haired skin (without too much normal skin) to abnormal crusted and alopecic skin. The blue circles are regions of thick crusting (primary or secondary lesion) representing a stage of disease that may differ from the green circle where skin is alopecic (primary or secondary lesion). The purple circle is the mucocutaneous junction which can be an important location for various disease processes.

Reference

1. Miller WH, et al. Diagnostic methods. In: Muller & Kirk's Small Animal Dermatology, 7th ed. Miller WH, Griffin CE and Campbell, KL, eds. Saunders-Elsevier, 2013:57-107.

Canine meningeal histiocytic sarcoma

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AHL Newsletter 2025;29(1):27.

A 6-year-old Belgian Malinois dog presented to the referring veterinarian with neurologic clinical signs that were localized to the spinal cord. MRI imaging revealed nodules in and around the spinal cord at multiple levels of the lower thoracic and lumbar spine. Large mononuclear cells were observed on cytologic examination of the cerebrospinal fluid. Postmortem histopathology confirmed an extensive infiltrate of neoplastic round cells that markedly expanded the meninges and subdural space, surrounded nerve roots, and infiltrated the parenchyma of the spinal cord (**Fig. 1A**). These cells were highly atypical, with round to reniform to bizarre nuclear outlines, frequent binucleation or multinucleation, 5-fold anisokaryosis, and >50 mitotic figures in ten high-power fields (40x/2.37mm2) (**Fig. 2**). Histiocytic sarcoma was the foremost consideration given the marked cellular pleomorphism, and this was confirmed by immunohistochemistry (IHC) for the marker Iba-1, with neoplastic cells demonstrating strong cytoplasmic reactivity (**Fig. 1B**). No information concerning metastasis or lymph node involvement was provided in the history.

Histiocytic sarcoma is a malignant neoplasm of histiocytic cell/dendritic cell/macrophage origin, which tends to be highly invasive and highly metastatic. As in this case, it can appear sporadically in any breed, however, Bernese mountain dogs are genetically predisposed. Commonly-reported primary locations for this neoplasm include the skin, joints of the limbs, and spleen. Histiocytic sarcoma of the central nervous system is less common, and most often presents as a focal mass rather than a diffuse meningeal infiltrate. Only two other infiltrative meningeal cases such as this have been seen at the AHL since 2015. Highly pleomorphic neoplastic cells are a typical feature of histiocytic sarcoma, however, confirmation of the diagnosis by IHC is still recommended, because marked atypia may also be observed in anaplastic round cell neoplasms.

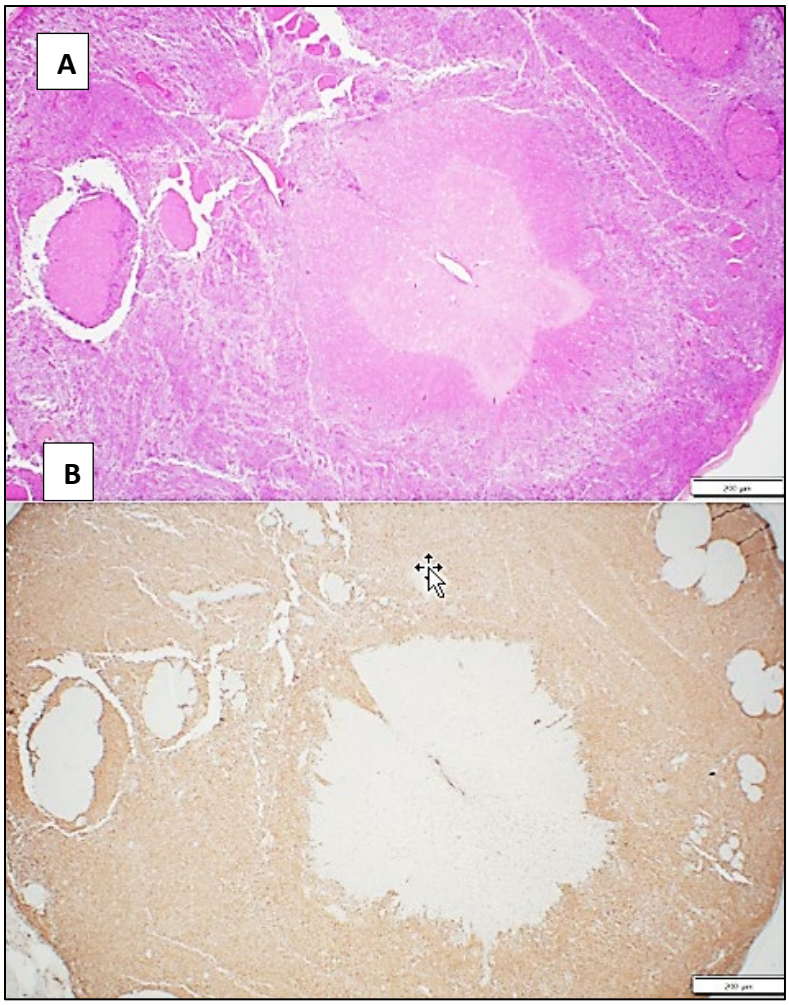

Figure 1. Canine, spinal cord. 1A. The meninges are markedly expanded by an infiltrate of neoplastic round cells which surround and invade the spinal cord and adjacent nerve roots. H&E stain.
1B. Neoplastic cells demonstrate strong reactivity (brown colour) for the histiocytic immunohistochemical marker Iba-1.

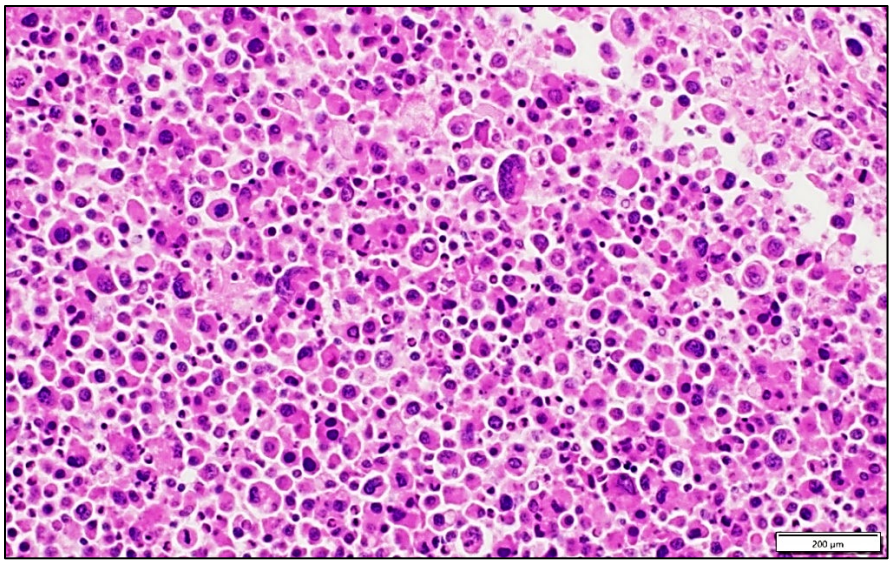

Figure 2. Canine, spinal meninges. Neoplastic histiocytic cells with atypia (variation in nuclear shape, size, and number). H&E stain.

Reference

1. Moore, PF. Canine and feline histiocytic diseases. In: Tumors in Domestic Animals, 5th ed. Meuten, DJ, ed. Wiley Blackwell, 2017:327-336.