Laboratory Services Division
Animal Health Laboratory

# AHL Newsletter

**AHL Newsletter, Volume 28, Number 3** **September 2024**

***In this issue:***



**AHL Newsletter**
September, 2024 - Volume 28, Number 3 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*

As we learned from the COVID pandemic, new diseases – particularly viral diseases – can arise and spread rapidly over wide geographical areas. Sometimes, other viruses that we believe we had under control can shift unexpectedly and cause new diseases or affect different species. This is the case with the B3.13 genotype variant of highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b. No one anticipated this virus's recent jump into cattle this year, causing systemic illness and mastitis in lactating dairy cattle in 13 US states at last count, 3 of which (Michigan, Minnesota, Idaho) border Canada: https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/hpai-confirmed-cases-livestock.
This variant also causes high mortality in poultry flocks and cats linked to affected dairy herds. Bovine veterinarians, diagnostic labs, animal health regulatory agencies and public health officials in Canada and the US have been meeting and sharing information frequently since the outbreak was confirmed, to understand how this viral variant spreads, and which species and animals are affected. The Canadian Animal Health Surveillance System (CAHSS) has done superb work in bringing together people from the aforementioned organizations, and in developing informational tools for people in the field. Bovine veterinarians who are attending cattle with compatible clinical signs are encouraged to contact their CFIA District Office and to submit samples (milk, urine, nasopharyngeal swabs) for HPAI testing to the closest CAHSN network laboratory. Companion animal veterinarians should consider HPAI on their list of differential diagnoses for cats with neurologic signs or sudden death, particularly those that live on or near dairy or poultry farms. CAHSS has developed an excellent fact sheet for HPAI testing in cats: https://cahss.ca/cahss-tools/document-library/highly-pathogenic-avian-influenza-A-H5N1-and-cats.

Let's hope for a quiet fall – but we should all be watchful nonetheless, so we can detect and limit the spread of significant disease outbreaks.

*Maria Spinato, Director*

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

# External testing at the Animal Health Laboratory

*Tim Pasma, Jennifer Zoethout*

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

AHL Newsletter 2024;28(3):3.

The AHL has over 1000 tests available to meet the needs of our clients. However, in some situations, the AHL sends samples to external laboratories for subcontracted or referral testing.

A test can be subcontracted when the AHL is unable to run a test for which it is accredited. For subcontracted tests, the AHL ensures that the external laboratory meets the accreditation of either the American Association of Veterinary Laboratory Diagnosticians or the ISO/IEC 17025 standard (General requirements for the competence of testing and calibration laboratories). The AHL contacts the subcontracted laboratory in advance to assess its quality program and competency as a laboratory.

In some circumstances, a client requests a test that is not offered at the AHL, or requests that a specific test be conducted at another laboratory. In these cases, the AHL can locate testing at another laboratory, and will send samples to that laboratory as a referral. For referral tests, the onus is on the submitting client to determine the quality of the external laboratory, and the AHL assumes no liability for the test results.

If you are looking for a test that is not listed in our test catalog, please contact the AHL and we will do our best to locate a laboratory that can perform the test for you. *AHL*

# OAHN Update – September 2024

*Mike Deane, Tanya Rossi*

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

This summer, the Ontario Animal Health Network has continued to create new species-specific reports and resources. Additionally, many OAHN-funded research projects have been started and completed. To view any of our network reports and research projects, go to OAHN.ca and navigate to the species you are interested in.

**OAHN Wildlife Network Project: Avian Influenza in Ontario Wildlife**

This project supported the testing of a variety of mammalian species for AIV in Ontario. During the project, AIV was detected in four additional species in Ontario (river otter, fisher, striped skunk, and raccoon). By comparing swab and brain samples for AIV detection, we were able to show that swabs are likely a reliable sample type for mammal testing, and we continue to rely on swabs as our main sample type for our ongoing AIV surveillance efforts. Read more here: https://www.oahn.ca/resources/oahn-wildlife-network-project-avian-influenza-in-ontario-wildlife/

**New Resources**

The OAHN Companion Animal network created a new factsheet on *Chlamydia caviae* in Guinea pigs. Find out about clinical signs, transmission, testing, treatment, and environmental considerations here: https://www.oahn.ca/resources/factsheet-chlamydia-caviae-in-guinea-pigs/

The OAHN Aquatics network has created two resources on viruses affecting freshwater fish. Click the links below for more:

- Cyprinid herpesvirus-1: Carp (Koi) pox
- Blotchy Bass syndrome virus

**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.

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

- OAHN survey: Ticks, HW, parvo, *Trich*
- H5N1 cows & cats
- FIP treatments
- New US dog import rules
- RHDV2: Back again
- Rabies update
- New & updated OAHN resources for vets!
- Promoting One Health @ your clinic
- A who's who of CA surveillance in ON

**Equine Network - https://www.oahn.ca/reports/equine-veterinary-report-q1-2024/**

- U.S. detections of HPAI in livestock
- BITS 'N SNIPS (or "things we talked about on the network call")
- Network member reports
- Syndromic and lab surveillance dashboard
- Equine research
- New! Ontario equine disease surveillance summary
- ResearchONequine

**Bovine Network - https://www.oahn.ca/reports/oahn-bovine-expert-network-quarterly-veterinary-report-q1-2024/**

- Global surveillance: Influenza A (H5N1) in U.S livestock
- Q1 bovine data from AHL
- Condemnations at Ontario abattoirs
- Digital dermatitis in feedlot cattle
- Bits and Bites

**Swine Network - https://www.oahn.ca/reports/swine-veterinary-report-q1-2024/**

- Novel Influenza A- H3N2 Cluster 2010.1 update
- Influenza A (IAV)
- Salmonella surveillance
- 19 new cases of Porcine Deltacoronavirus (PDCoV) and 7 new Porcine Epidemic Diarrhea virus (PED) cases in Q1 2024
- OAHN Veterinary clinical impression survey veterinary comments
- Laboratory Diagnostic Reports
- Ontario slaughter statistics
- International disease topics of interest summary

**Small Ruminant Network - https://www.oahn.ca/reports/small-ruminant-veterinary-2024-jan-mar-q1-review/**

- Highly pathogenic Avian Influenza in U.S. livestock
- Q1 2024 Animal Health Laboratory case data
- Provincial slaughter & condemnation data
- Input to the Sheep code of practice open until July 25
- Small ruminant research & resources

# Staff highlights

Mrs. Kimani Rutherford, AHL Bacteriology Team Lead, has been awarded the University of Guelph President's **Exemplary Staff Service** award in the category of Innovative Leadership. Kimani facilitates a positive and inclusive work atmosphere through effective communication, while ensuring a high standard of excellence. She implemented a comprehensive six-month training program geared towards addressing the team's collective needs that had a positive impact on her team's motivation and skill development. When the team was faced with an unexpectedly busy week, she made team appreciation a priority, organizing coffee and treats to boost morale and acknowledge everyone's efforts. By celebrating team members' efforts and contributions, Kimani fostered a positive and supportive work team culture in her unit.
We are glad that Kimani's contributions have been recognized by the President of the University of Guelph. Congratulations Kimani!

Dr. Đurđa Slavić, AHL Bacteriologist, has been awarded the prestigious **2024 Laboratorian of the Year Award** by the Canadian Animal Health Laboratorians Network (CAHLN). In addition to her workload as AHL Bacteriologist, she obtained a 5-year grant (2020-2024) from the US-FDA to participate as the only Canadian veterinary diagnostic laboratory in the Vet-LIRN network. This network supports the development of whole genome sequencing (WGS), and the collection of antimicrobial resistance (AMR) data. She is an enthusiastic supporter of One Health initiatives through her collaboration with the Public Health Agency of Canada to include antimicrobial susceptibility data capture for veterinary isolates into AMRNet. Dr. Slavić is currently the Canadian Provincial representative on the AAVLD (American

Association of Veterinary Laboratory Diagnosticians) Executive Board, a member of the WAVLD (World Association of Veterinary Laboratory Diagnosticians) Executive Board and also a member of the scientific organizing committee for the next WAVLD meeting to be held in Calgary in 2025.

All of us at the AHL consider Đurđa well-deserved of this exceptional award which is bestowed on an outstanding individual who has made a significant contribution to animal health laboratory medicine in Canada. Congratulations Đurđa!

# Presumptive polioencephalomalacia in beef cattle: A deceptive etiology

*Felipe Reggeti, Van Mitchell*

*Animal Health Laboratory, University of Guelph, Guelph, ON (Reggeti); Metzger Veterinary Services, Linwood, ON (Mitchell).*

AHL Newsletter 2024;28(3):8.

Steers from a feedlot facility in Ontario were examined for neurological signs consisting of ataxia, blindness, head pressing and recumbency. A new case was reported every 2-3 days for a total of 12 animals affected over a period of 3 weeks. The animals weighed approximately 500 kg, and the dry matter intake (DMI) was estimated to be around 13 kg. They received a total mixed ration (TMR) consisting of haylage, wheatlage, oat hulls, high moisture corn, corn silage and dry distillers grain. Some of the feed was "spoiled" and the wheatlage was 2 years old and sour smelling. The first 2 affected animals died before a veterinarian was involved in the case, and postmortem examinations were not performed. However, since the clinical signs were compatible with polioencephalomalacia (PEM), 6 g of 99% thiamine/head/day was added in the feed. Since cases continued to occur, parenteral injections of thiamine were initiated.

Excessive sulfur intake is an important risk factor for the development of PEM; therefore, water and feed samples were submitted for analysis. The feed contained 0.35% sulfur on a dry matter basis. A water sample collected from a water trough contained 5,109 ppm sulphate (equivalent to 1,703 ppm sulfur), and the sulfur content on a DMI basis was estimated to be 0.65%. With this information, the total sulfur concentration (TSC) relative to DMI (0.35% feed + 0.65% water) was 1%. Since the recommended maximal tolerable level of total sulfur in the diet for cattle is 0.3-0.5% dry matter (depending on amount of forage fed), a presumptive diagnosis of PEM due to excessive sulfur intake was suggested.

To confirm whether the water was the source of excessive sulfur (as there was no sulfur smell to the water), another sample was collected directly from the tap. This sample was submitted to a different laboratory, and the result was 37.9 ppm sulfate (~12.6 ppm sulfur). The significant discrepancy of the results between the 2 water samples raised concerns for laboratory error or contamination. The troughs were routinely washed on a weekly basis, and contamination with a sulfur-containing substance appeared to be unlikely. It was then discovered that the first water sample was collected in a clear sterile container used to test for the presence of coliform bacteria in drinking water, and some of these containers have sodium thiosulfate added to save laboratory preparation time and expense. Interestingly, the other element reported to be somewhat elevated in the first water sample was sodium (425 ppm), consistent with presumptive addition of sodium (thio)sulfate.

After adjusting the TMR containing 4 different fermented ingredients (some of poor quality), no additional cases occurred. Out of the 12 affected animals, only the two that were not treated with thiamine died, while the remaining 10 animals fully recovered. This case illustrates the importance of identifying the specific etiology in cases of PEM. Based on the initial analyses, a diagnosis of sulfur-related PEM could have been made; however, the sulfur content in the water was in fact acceptable and the root of the problem was not excessive sulfur consumption. It was speculated that the inadequate diet might have stimulated over-growth of thiaminase-producing bacteria, or caused impaired thiamin absorption/utilization. This was supported by response to thiamin administration, and correction of the

problem after adjusting the TMR. *AHL*

Reference
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# Johne's Disease (*Mycobacterium avium paratuberculosis*) infection in goats

*Siobhan O'Sullivan, Lisa Gordon*

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

AHL Newsletter 2024;28(3):9.

Multiple goats from a small herd developed chronic diarrhea, progressive loss of body condition, and lymphadenopathy. On postmortem examination, lymph nodes appeared caseonecrotic, the mesenteric vasculature was dilated, and the wall of the intestine was segmentally thickened. Histopathology revealed granulomatous enteritis and lymphadenitis, with caseous necrosis and mineralization in the lymph node. With modified acid fast staining, a few positively-staining organisms were visualized in macrophages. Fecal samples were positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) by PCR (cycle threshold [Ct] values 29-35), confirming the diagnosis of Johne's disease.

Clinical signs of Johne's disease are secondary to the granulomatous enteritis produced by MAP infection. Intestinal malabsorption of protein results in diarrhea, cachexia, hypoproteinemia, tricavitary effusion, and eventual mortality (**Figs. 1,2**). Common gross lesions include ileocecal and mesenteric lymphadenopathy, thickening of the mucosa of the ileum and colon into elevated ridges, and thickened, white intestinal lymphatics (lymphangitis) (**Figs. 1,2**). These lesions can be more subtle and variable in small ruminants than in cattle. Caseous necrosis of the lymph nodes with mineralization is also reported sporadically in infected small ruminants. Histologically, acid fast organisms can be abundant (multibacillary) or sparse (paucibacillary) in macrophages, necessitating a thorough scanning of the tissues to identify the diagnostic bacteria (**Fig. 2**).

Ruminants are often infected while young, through milk or the fecal-oral route, and can remain asymptomatic for 2-5 years while shedding the organisms through feces. Mature animals eventually develop weight loss and diarrhea. These long-term subclinical individuals act as reservoirs of infection for other animals, making Johne's difficult to control. Since it is not effectively treatable, management of Johne's focuses on long-term preventative strategies: removing animals that test positive; acquiring replacements from disease-free stock; and reducing the risk of fecal-oral spread to young animals through sanitation practices. *AHL*

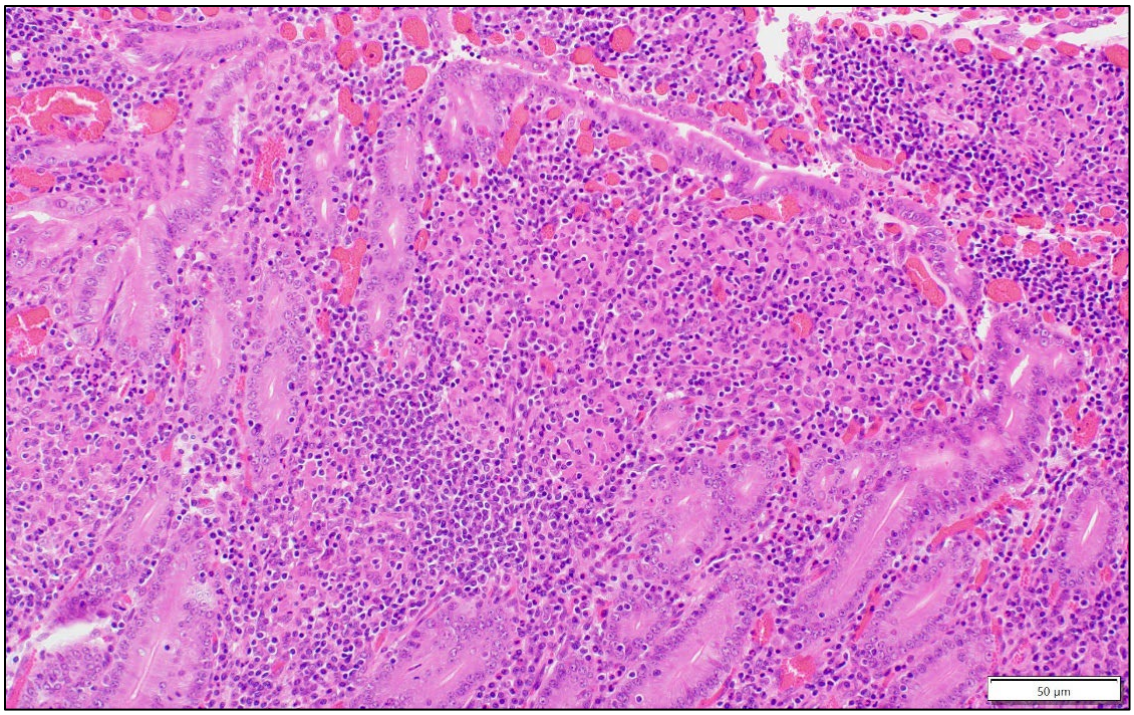


**Figure 1.** Goat, small intestine. Aggregates of macrophages are present in the villus lamina propria. H&E stain.

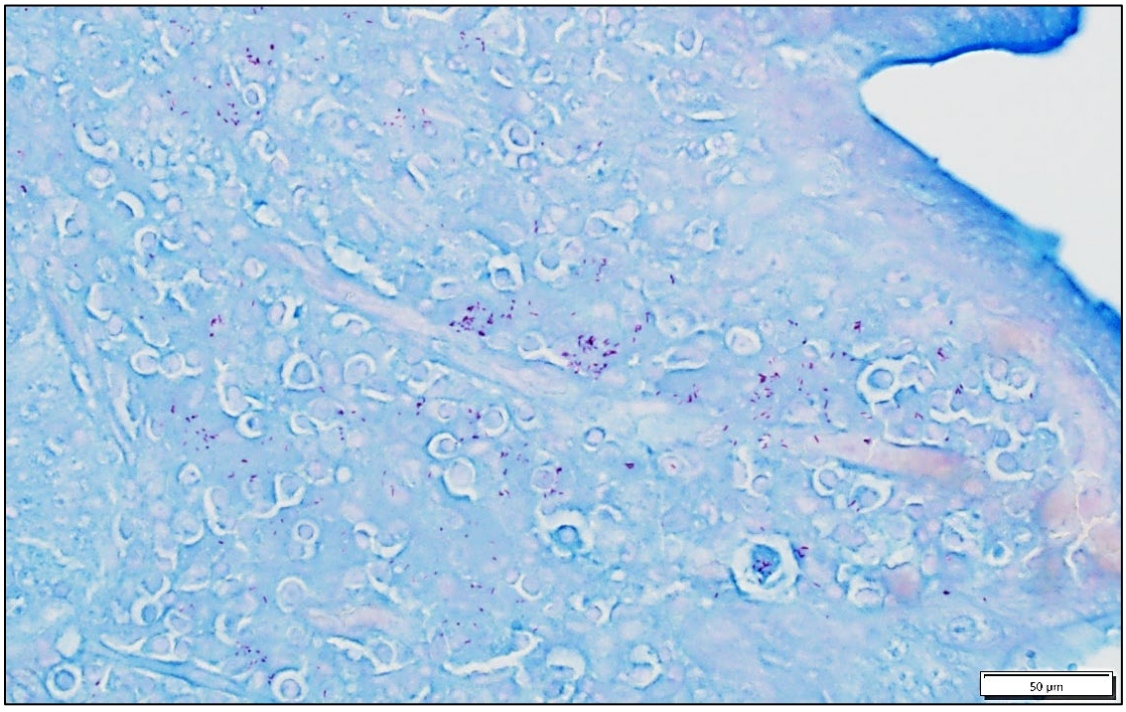


**Figure 2.** Goat, small intestine. Modified acid fast-positive bacteria are present in the cytoplasm of macrophages. ZN stain.

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3. Grieg A. Johne's disease in sheep and goats. In Practice. 2000;22(3):146-151.

# Lancet fluke in the liver of a bison

*Jacob Avula, Siobhan O'Sullivan, Tim Pasma*

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AHL Newsletter 2024;28(3):11.

*Dicrocoelium dendriticum*, otherwise known as lancet fluke or small liver fluke, has been reported in sheep, goats, cattle and a miniature horse in Ontario. A detailed description of the cases, including the life cycle, was provided by previous authors. The occurrence of this parasite in a bison is herewith reported.

The bison, which was located in Southwestern Ontario, was taken to the butcher. The meat inspector noticed tracts in the liver and wanted the liver examined and the parasite identified. Few adult parasites were recovered from the bile ducts of the liver at AHL, and the parasites were identified as *Dicrocoelium dendriticum*, based on the morphological characteristics (**Fig. 1**). *AHL*

**Figure** 1. *Dicrocoelium dendriticum*.

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# Swine abortion diagnosis: Are you stressed?

*Emily Brouwer, Tim Pasma*

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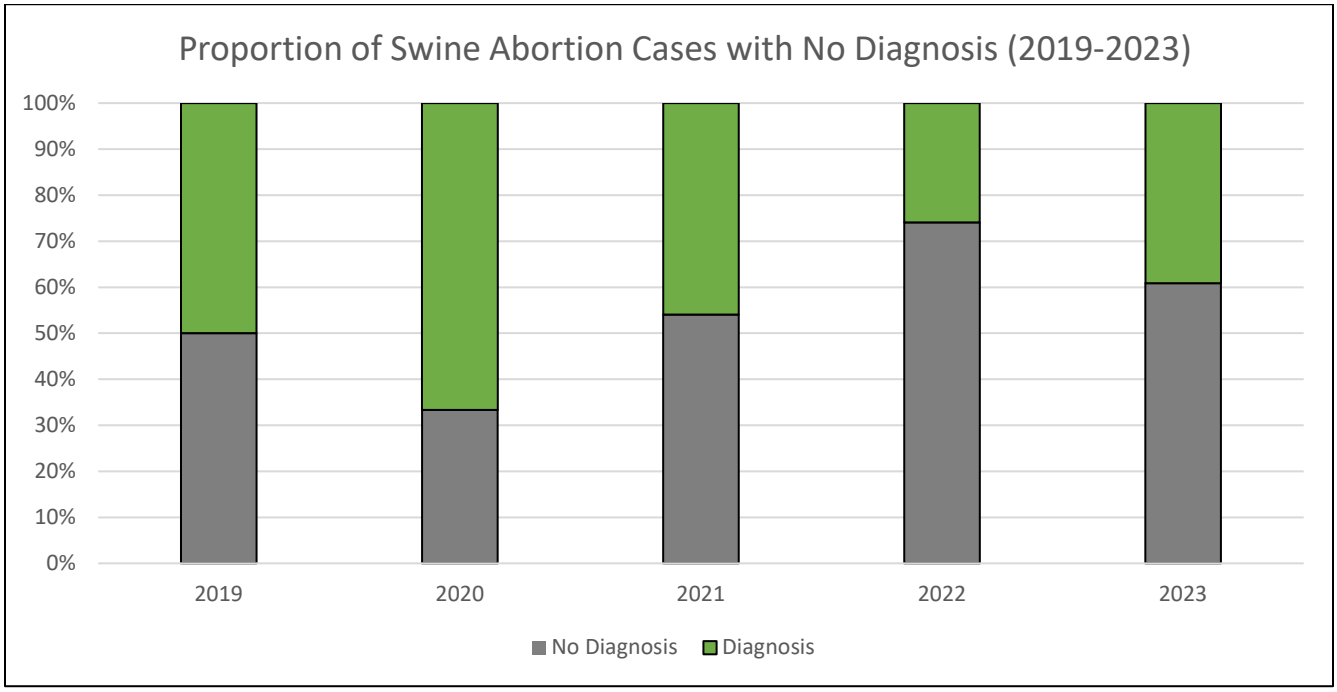
AHL Newsletter 2024;28(3):12.

Swine veterinarians and pathologists are frequently asked to identify the cause of reproductive failure/abortion in swine. Fetuses that are submitted to the Animal Health Laboratory are examined by a pathologist, and a routine set of tissues are sampled for histology, bacterial culture, and virology testing. The pathologist identifies morphologic changes, either grossly or histologically, and integrates these findings with the results of ancillary testing. A relatively high proportion of these cases will have no specific diagnosis: percentages of non-diagnostic cases have ranged from 25.9 % to 66.7 % over the last five years (**Graph 1**).




**Graph 1.** Proportion of swine abortion cases with no diagnosis (2019-2023)

Although receiving a report without an etiologic diagnosis is inherently frustrating for both the submitting veterinarian and the pathologist, it is important to recognize that not all causes of abortion are infectious, and not all causes of abortion will cause grossly or histologically detectable lesions.

Non-infectious causes of abortion include a nebulous collection of factors including seasonal effects, ambient temperatures, stress factors, and toxic substances. One such example is heat stress, where high ambient temperatures or increased humidity lead to redistribution of blood flow in the dam at the expense of uterine and placental perfusion, thus increasing the risk of pregnancy failure. In addition, heat stress at

the time of breeding and implantation can lead to embryonic mortality, increased return to estrus, and small litter sizes. In a similar vein, seasonal infertility in pigs occurs in late summer to early autumn and is associated with photoperiod and high temperatures. This physiologic process is thought to be a vestige of seasonal breeding in ancestral wild boars, and aberrations in progesterone—essential for maintaining pregnancy—linked to the alternation in daylight.

Stress in the dams is also linked to pregnancy failure, and encompasses various environmental and social factors, including movement and comingling. The pathogenesis of pregnancy loss secondary to stress is poorly understood, but proposed mechanisms include cortisol-mediated effects on the pituitary gland and subsequent downstream effects on corpora lutea, as well as increased basal body temperature, and increased contractility of the uterus in times of excitation.

Maternal illness in the sows can also result in abortion, with no appreciable lesions in the fetuses or fetal tissues. Assessing the sows at the time of an abortion outbreak is critical, and evidence of systemic disease in the sows should be investigated concurrently with routine abortion diagnostic testing. In some circumstances (e.g., influenza A virus infection), maternal illness does not result in viremia and fetal infection, but rather the maternal systemic inflammatory response disrupts the hormonal balance required for maintenance of pregnancy.

Various toxins are associated with failure of pregnancy. One toxin of note, carbon monoxide (CO), can lead to abortion and stillbirth with no clinical signs in the sows, as there is greater affinity of carbon monoxide for fetal hemoglobin versus maternal hemoglobin. CO toxicosis in fetuses results in cherry red discolouration of the tissues, and is confirmed with carboxyhemoglobin testing in fetal thoracic fluid. Mycotoxins are also implicated in porcine failure of pregnancy, though the experimental evidence does not implicate any particular mycotoxin in abortion, and mycotoxin ingestion resulting in abortion in an otherwise healthy sow is considered rare. The notable exception amongst mycotoxins, however, is zearalenone. This estrogenic mycotoxin tends to impact conception and litter size, rather than causing abortion.

The unifying trait for the above-listed conditions is that (with the exception of carbon monoxide poisoning) the pathologist will see no characteristic gross or histologic lesions, the battery of ancillary tests will identify no significant pathogens, and a report will be sent off with the diagnosis of “idiopathic abortion”. The diagnostic workup for common causes of abortion includes PCR testing for PRRSV, PCV 1, 2 and 3, parvovirus, and potentially, *Leptospira* spp. Bacterial culture is also performed on placenta, lung and stomach content in order to identify possible infectious causes. Negative results in these cases, while often frustrating, are important pieces of information in ruling out infectious causes.

In order to maximize the diagnostic utility of the submitted specimens, the Animal Health Laboratory recommends submitting up to three litters of aborted fetuses, taking care to include placentas and keeping the fetuses chilled (or frozen, if necessary). PCR and bacteriologic testing should be pooled by litter. A thorough history, taking note of sow health, is always appreciated. *AHL*

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# Porcine circovirus-2 (PCV-2) associated disease in swine

*Siobhan O'Sullivan*

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

AHL Newsletter 2024;28(3):14.

Multiple weaner pigs from a small swine operation presented with neurologic clinical signs that included convulsions, hind limb ataxia, and dog sitting. On postmortem examination, the cranioventral lung lobes were dark red and firm. Histopathology confirmed a lymphocytic meningoencephalitis, as well as granulomatous and neutrophilic interstitial pneumonia. In the ileum, submucosal Peyer's patches were notably depleted of lymphocytes (**Fig. 1**), and contained rare macrophages with intra-cytoplasmic, botryoid viral inclusion bodies (**Fig. 2**). Fresh lung from this submission was positive for porcine circovirus-2 (PCV-2) by PCR (cycle threshold [Ct] value 13.38), confirming PCV-2 as the cause of neurologic signs and mortality.

PCV-2 associated disease (PCVAD) can produce a variety of clinical signs, gross lesions, and histologic lesions, and there can be obfuscating overlap with other viral infections such as porcine arterivirus (PRRSV). Grossly, lymphadenopathy and pneumonia are suggestive of PCV-2 infection, and some of the most characteristic histologic lesions of PCV-2 infection are granulomatous interstitial pneumonia, lymphocytic meningoencephalitis, granulomatous lymphadenitis, and lymphoid depletion, with diagnostic viral inclusion bodies in macrophages (**Fig. 1**). While PCV-2 is a common diagnosis for cases coming through the AHL, particularly as a contributor to the porcine respiratory disease complex, it is uncommon to see the inclusion bodies on histopathology, presumably as a consequence of the stage of infection. Pigs can be chronic carriers of PCV2, therefore detection of the virus by PCR, especially at a high Ct value, is not sufficient for a diagnosis of PCVAD; compatible lesions, with a low Ct value, or demonstration of the virus in tissue by immunohistochemistry, are the standard for confirming the diagnosis.

PCV-2 is also the causative agent of porcine multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), and abortion. The virus is transmitted between pigs by contact with infectious secretions (respiratory, nasal, ocular etc.) as well as through urine and feces, and possibly through fomites (**Fig. 2**). Vaccination and biosecurity protocols are advisable for prevention and control.

*AHL*

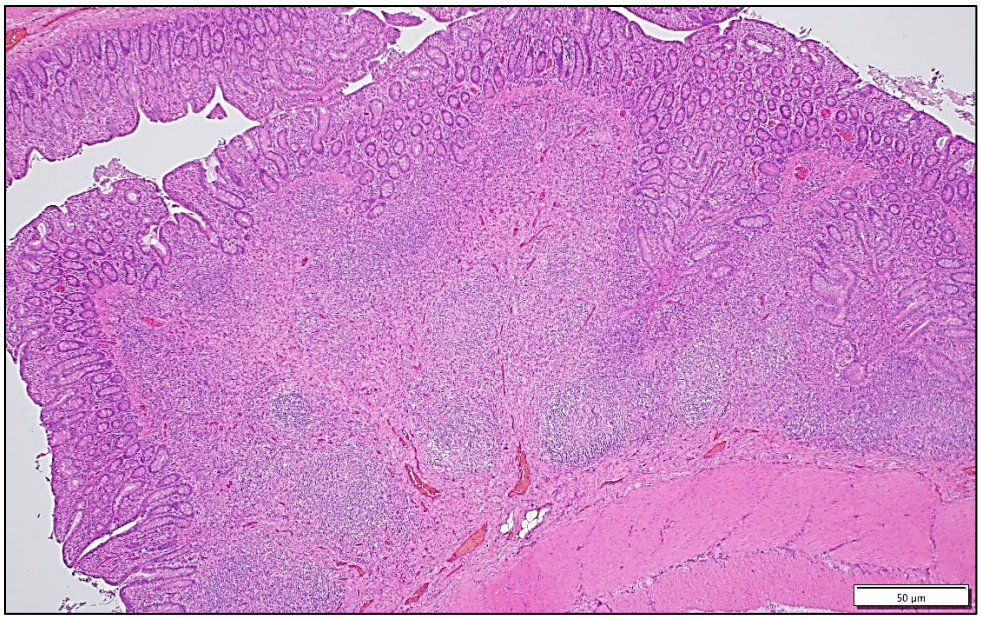


**Figure 1.** Porcine ileal Peyer's patch. Submucosal Peyer's patches are indistinct due to loss of cellularity. H&E stain.

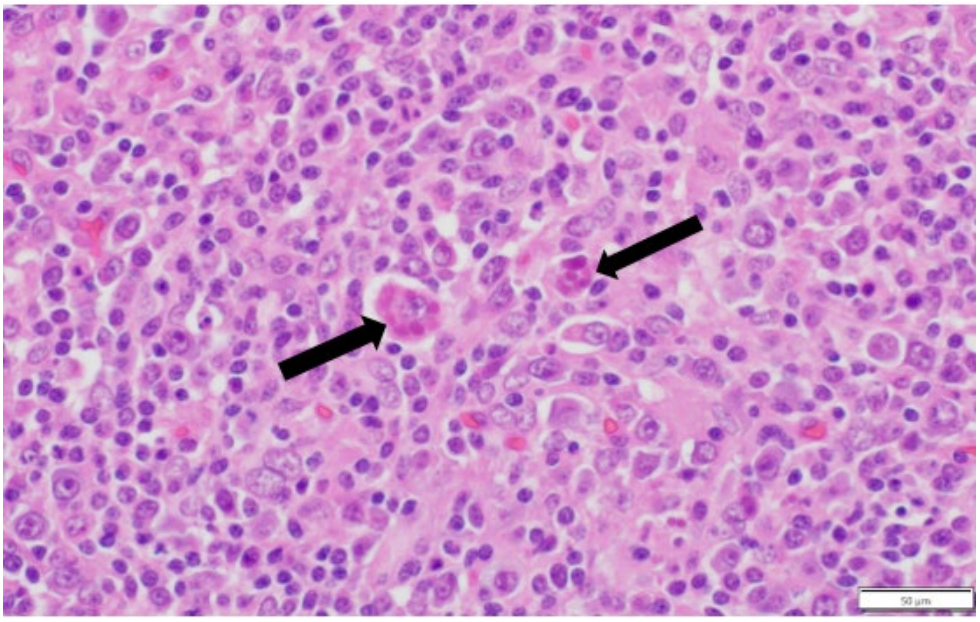

**Figure 2.** Porcine ileal Peyer's patch. Macrophages with intracytoplasmic botryoid viral inclusion bodies (arrows). H&E stain.

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# Columnaris disease in rainbow trout

*Hein Snyman, Lowia Al-Hussinee, Ledger, Lisa, Hugh Cai*

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AHL Newsletter 2024;28(3):16.

*Flavobacterium columnare* is the causative agent of "columnaris disease", a significant ulcerative pathogenic bacterial infection that affects freshwater fish species. It is of particular economic significance in freshwater aquaculture, where the disease can result in devastating losses, especially in rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis* sp.), and catfish (*Ictalurus* sp.).

The disease was first described in the early 1900s, and the causative bacterium has since then undergone numerous name changes, initially starting out as *Bacillus columnaris* and having been named due to its tendency to form column-like masses/colonies of bacteria when necrotic surface debris was reviewed on cytological examination (**Fig. 1**). Following that, the bacterium quickly transitioned through a variety of names, including *Chondrococcus columnaris*, *Cytophaga columnaris,* and *Flexibacter columnaris,* before finally settling on *Flavobacterium columnare*.

Although "columnaris disease" was historically thought to be caused by this singular species, more recent genetic work has resulted in the identification of four discrete genetic groups referred to now as "columnaris-causing bacteria (CCB)", with these groups also exhibiting some specific species associations:

*F. columnare* (most common isolate in rainbow trout)
*Flavobacterium covae* sp. nov. (most common isolate in catfish)
*Flavobacterium oreochromis* sp. nov (most common isolate in tilapia)
*Flavobacterium davisii* sp. nov. (multiple different species)

Early infections can be non-specific with fish initially presenting with decreased feed intake, lethargy, a tendency to congregate or swim closer along the water surface, and increased opercular movement (gilling activity). Clinical symptoms quickly transition to the more characteristic signs of this disease which include patchy skin and fin discolouration with worsening erosion and eventual deep ulceration (**Fig. 2**). Although any external surface can be affected, changes are often most prominent along the dorsal fin and trunk, but other commonly-affected areas include the mouth/lips and gill arches. Histologically, lesions tend to be quite necrotizing with ulcerated surfaces and necrotic tissue being widely covered and permeated by dense mats of pale basophilic filamentous bacteria (**Fig. 3, 4**).

Mortality is usually associated with derangement of osmotic homeostasis following the loss of the integrity of the skin, however, hypoxia associated with significant loss of functional gill tissue can also contribute. Surface abrasion or pre-existent cutaneous injuries can play a significant role in the initial colonization by the bacterium, while stressors such as water temperature, low dissolved oxygen, or other aberrant aqueous environmental factors tend to initiate outbreaks and worsen disease progression. Flavobacteria are common aqueous environmental organisms, and as such, environmental contamination can significantly drive continued horizontal spread between fish.

Diagnosis of columnaris disease is based on a combination of clinical signs, wet mount cytology, gross and histological lesions, and positive bacterial isolation and identification (**Fig. 5**). However, specialized

culture media is required for the isolation of Flavobacteria, and its availability is usually limited to laboratories that routinely deal with aquatic animal submissions. Given that isolation can sometimes also be difficult, the AHL has also recently validated a PCR assay for the identification of *F. columnare* in tissue samples (e.g., gill, skin, internal organs) which can further aid in confirmation. As with all PCR tests, positive results must be interpreted in conjunction with clinical signs, biopsy wet mount examination, and compatible gross and histological changes. Since initiating the *F. columnare* PCR assay in April 2024, we have tested 17 cases by PCR. Preliminary data shows that most cases have consistent results among the different tests, and that *F. columnare* PCR-positive results were significant even when the Ct value was as high as 40 (**Table 1**). *AHL*

**Table 1.** Comparison of F. columnare PCR with histology, gill and skin biopsy and isolation (NBP = No bacterial pathogens; NBG = No bacterial growth; ND = No data).

| **Sample ID** | **Species** | ***F. columnare* PCR cycle threshold** | **Histology and gill/skin biopsy** | ***F. columnare* isolation** |
|---|---|---|---|---|
| 24-070742 | Walleye | 29.03 | Columnaris disease (ulcerative dermatitis and stomatitis) | *F. columnare* 2+ (gill, kidney) |
| 24-060669 | Rainbow trout | 34.1 | Columnaris disease (gill necrosis) | *F. columnare* 2+ (gill, kidney) |
| 24-067929 | Rainbow trout | 35.01 | Columnaris disease and Bacterial gill disease | *F. columnare* 2+ (gill, kidney) |
| 24-050206 | Rainbow trout | 37.27 | No obvious bacterial infection | *F. psychrophilum* 1+ (kidney) |
| 24-050163 | Rainbow trout | 39.22 | Columnaris disease (ulcerative dermatitis) | *F. columnare* 2+ (gill, kidney) |
| 24-060685 | Rainbow trout | 40 | Columnaris disease (suspected) | *F. columnare* 3+ (gill) |
| 24-063539 | Rainbow trout | 40 | Columnaris disease (gill necrosis), Bacterial gill disease | NBP |
| 24-059163 | Rainbow trout | Not detected | No columnaris disease | NBP |
| 24-059171 | Rainbow trout | Not detected | No columnaris disease | NBG |
| 24-059178 | Rainbow trout | Not detected | No columnaris disease | *F. columnare* 3+ (gill) |
| 24-060136 | Arctic char | Not detected | Filamentous bacterial and oomycetes dermatitis | *F. columnare* 2+ (gill, spleen) |
| 24-060649 | Rainbow trout | Not detected | Columnaris disease (gill necrosis) | ND |

| | | | | |
|---|---|---|---|---|
| 24-065709 | Rainbow trout | Not detected | ND | ND |
| 24-065724 | Rainbow trout | Not detected | ND | No *F. columnare* isolated |
| 24-068407 | Rainbow trout | Not detected | ND | No F. columnare isolated |
| 24-070348 | Rainbow trout | Not detected | Bacterial gill disease | Aeromonas sobria 2+ |
| 24-070695 | Splake | Not detected | Furunculosis | A. salmonicida 2+ |

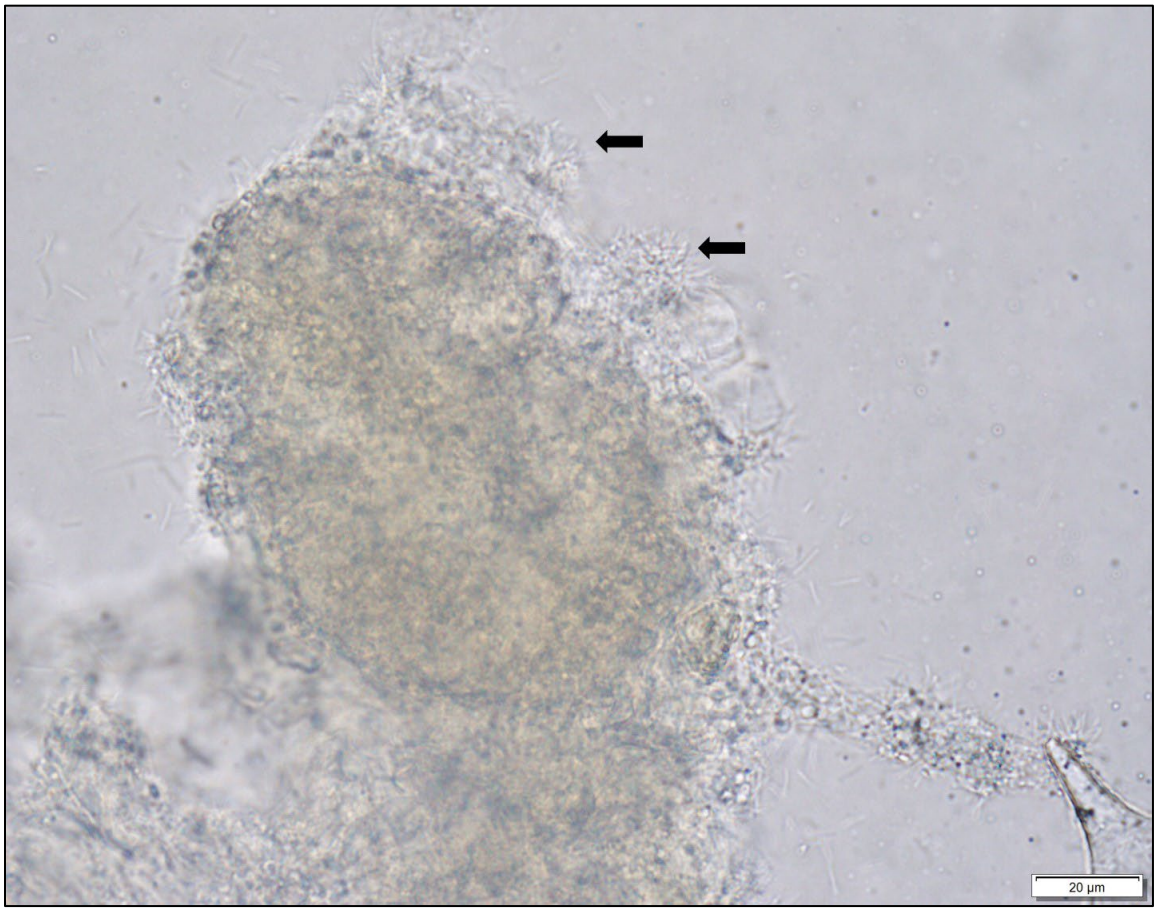


**Figure 1.** Wet mount cytology from the skin of a rainbow trout affected with columnaris disease exhibiting column-like stacked mats of slender filamentous bacteria (arrows).


**Figure 2.** Rainbow trout fish with cutaneous ulceration along the lateral body wall, characteristic of columnaris disease (arrow).

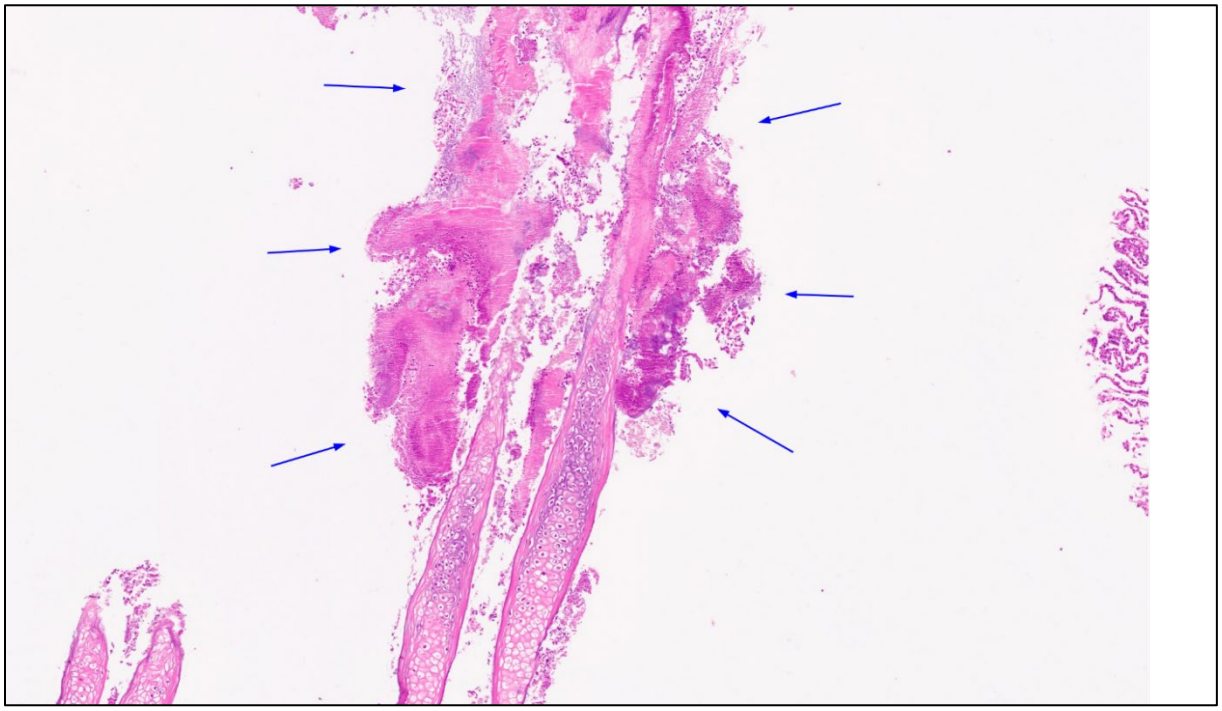

**Figure 3.** Histopathology of gill with regional necrosis of the gill filaments and abundant necrotic debris (arrows). H&E stain.

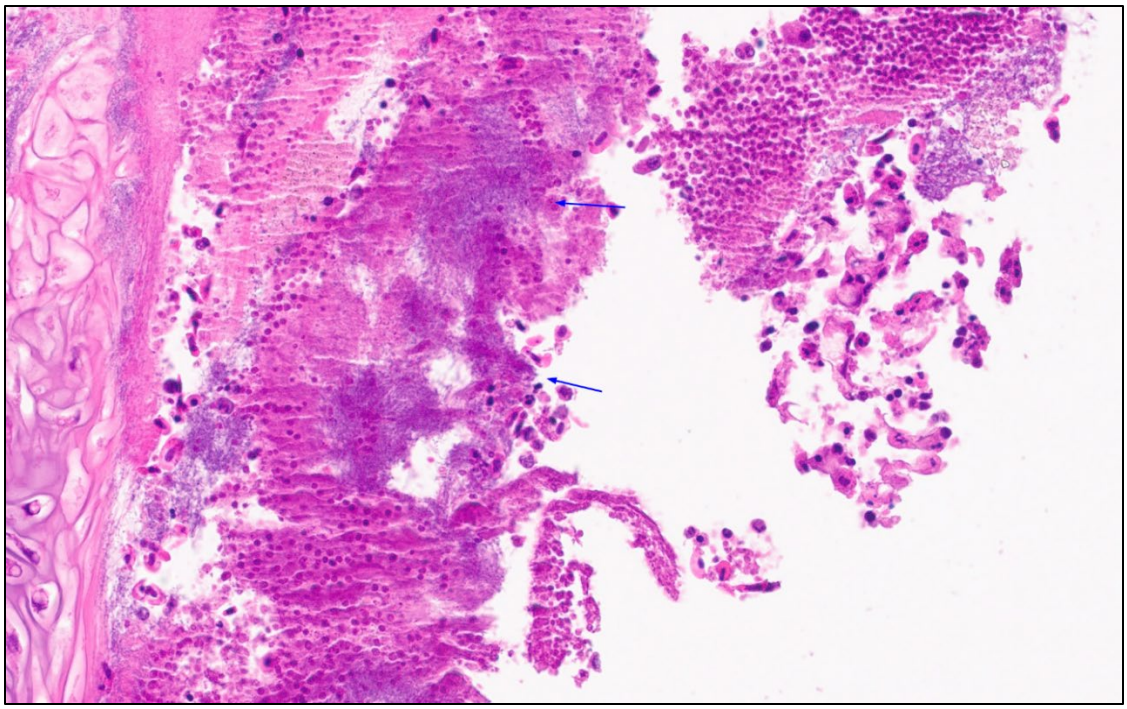

**Figure 4.** Higher magnification of the gill with necrotic debris being widely permeated by a dense meshwork of filamentous bacteria (arrows). H&E stain.

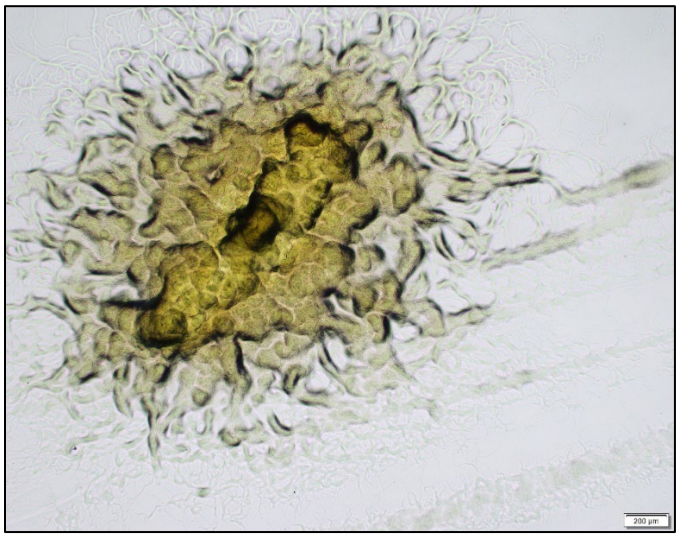

**Figure 5**. Bacterial culture plate morphology of a *F. columnare* colony exhibiting characteristic flat rhizoid structure with irregular margins. Also note the characteristic yellow pigmentation of the colony that can also afford yellow tissue discolouration in affected fish.

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# Chronic progressive lymphedema in a draft horse

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AHL Newsletter 2024;28(3):21.

Following the euthanasia of a 7-year-old female Clydesdale horse with progressively worsening lameness and weight loss, the animal was submitted to the AHL for postmortem. On gross examination, markedly expanding and encircling the pastern, fetlock and extending up the cannon bone, the skin surface of all four limbs was massively thickened by multifocal to coalescing, firm/fibrous, variably-sized (2-3 mm up to 5 cm) raised, partially haired or alopecic nodules. There was frequent dermal ulceration and deep folds filled with purulent exudate and crusted material (**Fig. 1**). The hoof surfaces were heavily cracked and irregular with an undulating and uneven contour. The tip of the tail was markedly expanded (to a 17 cm diameter) by firm, fibrous tissue with an alopecic, black dermal surface, and few 1-2 cm surface ulcers. There was mild bruising and excoriation/ulceration with flaky yellow crusting of the haired skin encircling the vulva, extending ventrally to the inguinal region and the medial surface of the thighs. CT imaging and sagittal sectioning of the limbs revealed a severe deep digital flexor tendinopathy of the left forelimb, multi-limb osteoarthrosis of the interphalangeal joints and pedal osteitis.

Microscopic lesions of the limbs, tail tip and perivulvar/inguinal regions included marked hyperkeratosis, surface necrosis and ulceration with formation of deep necrotic crevasses between skin folds and marked superficial to deep dermal fibrosis. There was prominent nested proliferation of superficial, middle and deep vascular plexi with thickening of vessel walls by smooth muscle hyperplasia, accompanied by thick rings of peripheral fibrosis. Lymphatic vessels were mildly to markedly dilated and tortuous with variable perilymphatic fibrosis and inflammation (**Fig. 2**).

Gross and histological findings were all compatible with a diagnosis of a severe case of chronic progressive lymphedema (CPL). Previous terms for this condition included “chronic pastern dermatitis” and “chronic proliferative pastern dermatitis”, but because of the clinical and histological similarity to the human counterpart “non-filarial chronic lymphedema” (or elephantiasis nostras verrucosa), the condition was renamed “chronic progressive lymphedema in horses”. CPL is a debilitating condition where there is buildup of lymph fluid in the lower legs, progressive swelling with associated skin folds, nodules and ulcerations. Secondary bacterial, fungal, or parasitic infections often complicate and aggravate the lesions and lead to advanced progression of this disease. Many affected horses also develop poor hoof growth. The dermal lesions can limit the horse’s movement and result in lameness due to the inappropriate alignment of the articular bones, which is the potential cause for the changes to the phalangeal bones and tendinopathy diagnosed in this case.

CPL is more prevalent in certain bloodlines, therefore, a genetic component is suspected. Belgian draft horses are the most frequently affected; however, other breeds including Shires and Clydesdales, Gypsy Cobs and Gypsy Vanners, Friesians, the American Belgian, German draft horse breeds, the Percheron, and other large breed draft horses have been documented to be diagnosed with CPL.

Dysfunction of the lymphatic system, progressive fibrosis of the tissues, and a disruption of the elastin matrix in the skin all appear to be components of the pathogenesis, however, the chronology of these changes and exact mechanisms of this disease remain uncertain. *AHL*

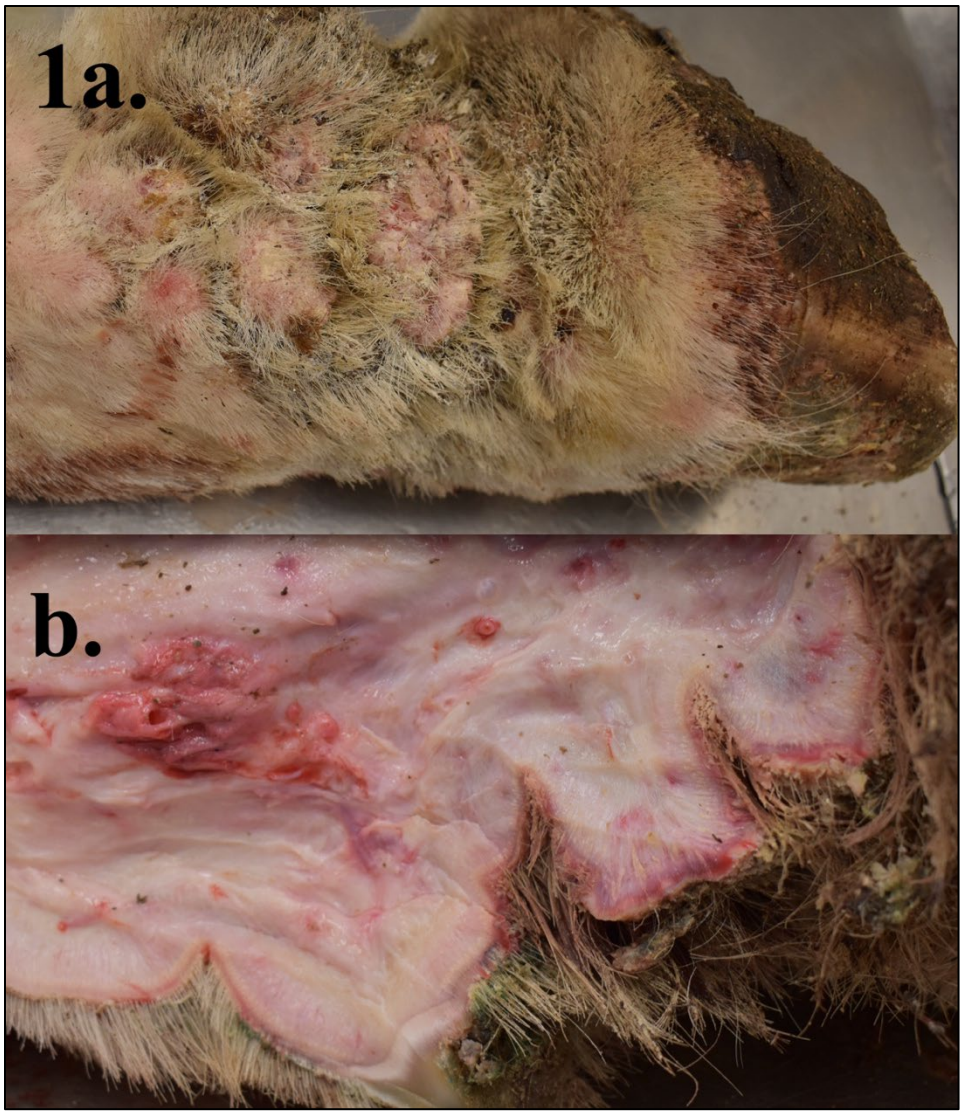


**Figure 1.** Gross lesions of the distal limb compatible with chronic progressive lymphedema.
**a.** Marked dermal thickening with multifocal to coalescing, firm, fibrous, partially-haired or alopecic nodules with frequent dermal ulceration, and deep folds filled with purulent exudate and crusts.
**b.** A cross section of the cutaneous nodules demonstrates marked dermal fibrosis.

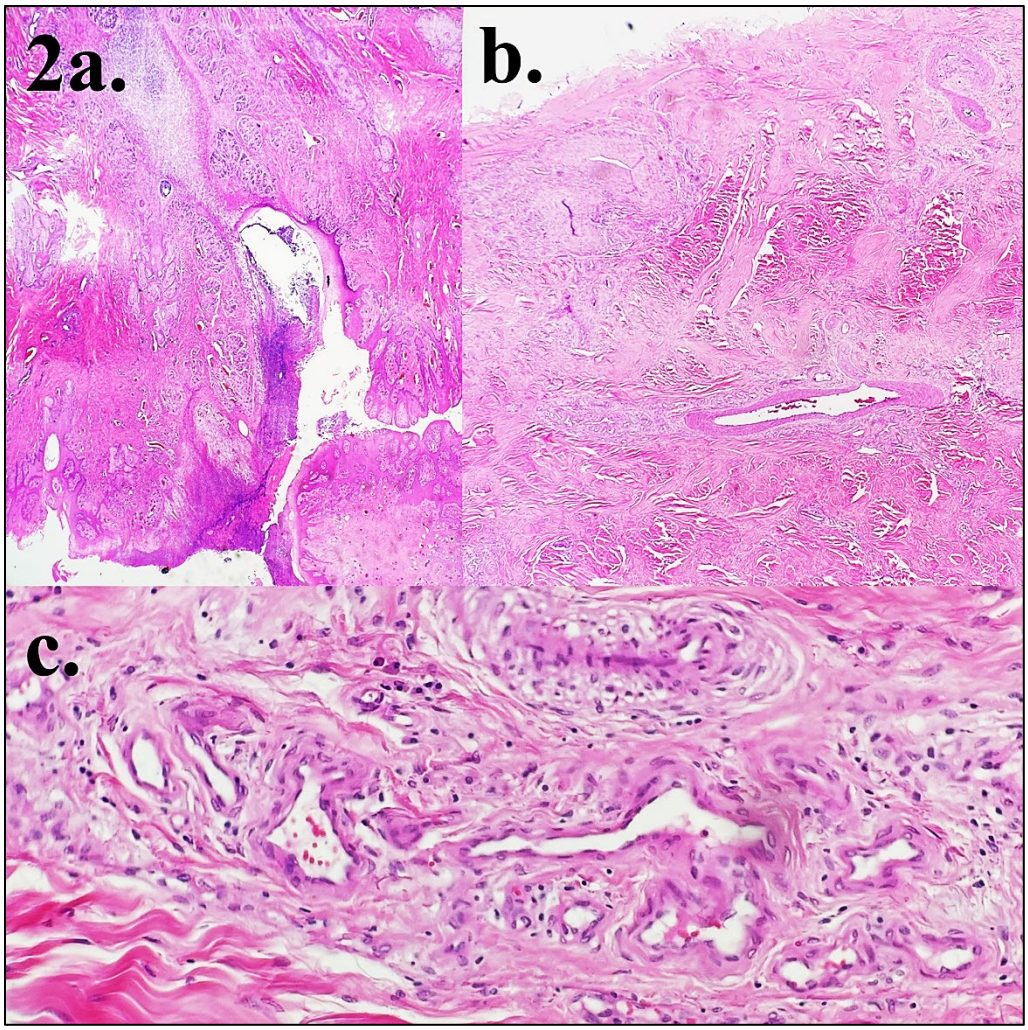


**Figure 2.** Histologic lesions compatible with chronic progressive lymphedema**.** H&E stain.
**a.** Hyperkeratosis with thick crusts and formation of deep necrotic crevasses between skin folds 10x. **B.** Marked deep dermal fibrosis and proliferation of nested vascular plexi with surrounding fibrosis. 20x. **C.** proliferative vascular nest with surrounding fibrosis and mild inflammation. 40x.

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# Sarcoptic mange in a dog

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AHL Newsletter 2024;28(3):24.

A three-year-old Labrador retriever crossbred dog presented in early autumn for erythema and pruritus that was unresponsive to courses of antibiotics and steroids. There was severe erythema of the legs, ventrum and groin with a few dried pustules on the ventral abdomen (**Fig. 1**). Skin cytology showed neutrophils and rare macrophages with no bacteria or acantholytic cells. Four skin biopsies were taken from the affected ventral abdominal skin, fixed in formalin and sent to the AHL for histopathology. Microscopically there was chronic hyperplastic superficial perivascular dermatitis with hyperkeratosis, intracorneal pustules and intralesional *Sarcoptes scabiei* mites (**Fig. 2**). The severe erythema and pruritus were therefore attributed to sarcoptic mange.

Canine sarcoptic mange is an intensely pruritic contagious skin disease caused by *Sarcoptes scabiei*, a burrowing epidermal mite. Transmission occurs by contact with an infected dog or a contaminated object or environment. Wild canids such as red foxes and coyotes can be infected, and can also serve as a source of infection through direct contact or contact with contaminated dens. *Sarcoptes scabiei* is relatively host specific; however, transmission can occur to non-host adapted species such as cats or humans, generally resulting in a self-limiting pruritus that appears as a rash in human skin. Pruritus is the main clinical feature of canine sarcoptic mange. Erythema, crusts, papules and alopecia are also seen, sometimes with excoriations secondary to self-trauma. In longstanding cases there may be widespread alopecia with lichenification and hyperpigmentation. The margins of the pinnae are often the first site involved; ventral trunk (both abdomen and chest), lateral elbow and face are other commonly-affected sites. Secondary lymphadenopathy, pyoderma and *Malassezia* dermatitis may be present. Clinical differential diagnoses include flea allergy dermatitis and superficial pyoderma. Diagnosis may be achieved by skin scraping or fecal flotation (to detect ingested mites and eggs). Mites can be difficult to find in skin scrapings, so if the clinical suspicion is high and these tests are negative, then empirical treatment or skin biopsy are useful for diagnosis. *AHL*

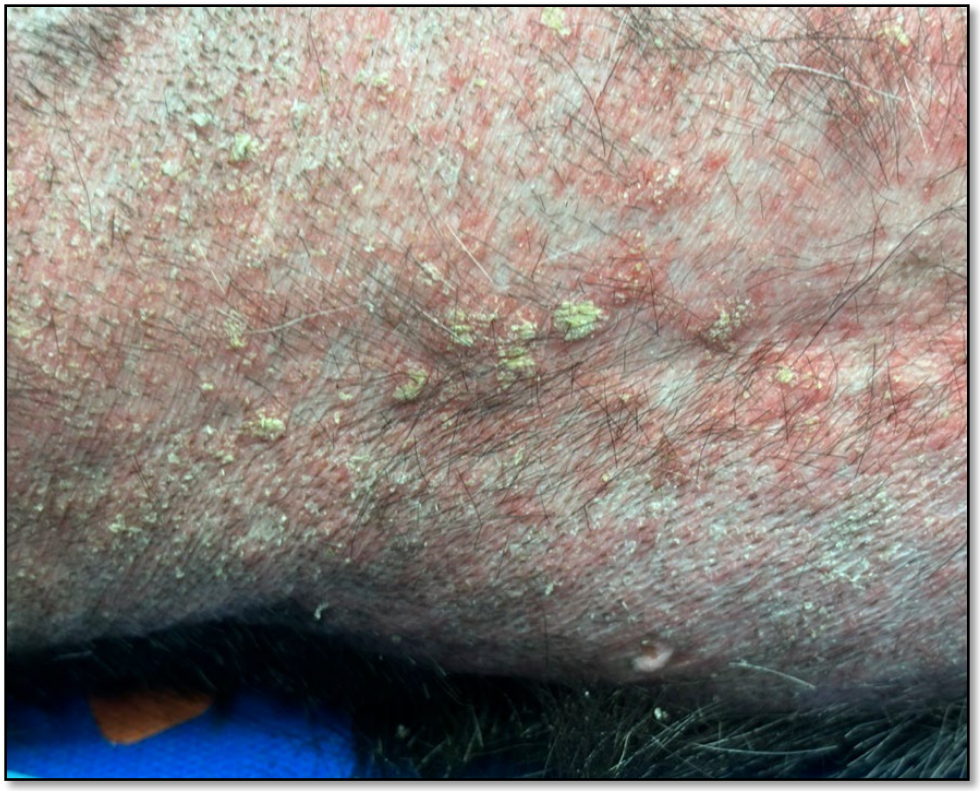

**Figure 1.** Sarcoptic mange. Erythema with crusts and multifocal dried pustules on the ventral abdomen

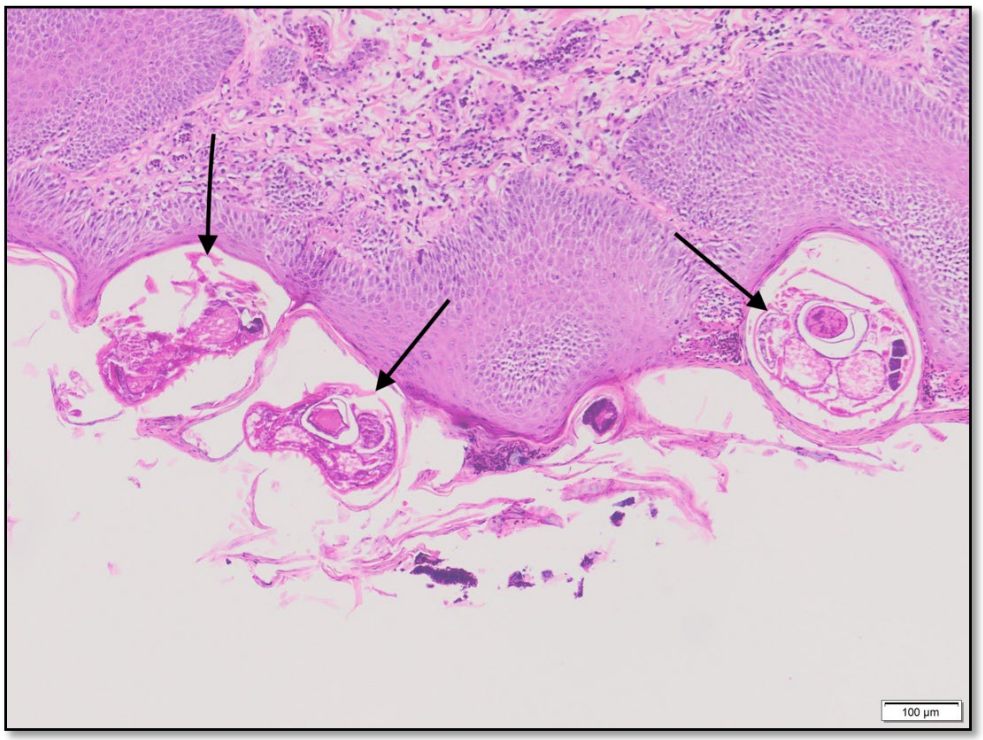


**Figure 2.** Histologic section of the skin. Embedded in the stratum corneum of the epidermis are several *Sarcoptes scabiei* mites (arrows). H&E stain.

# New CLSI guidelines – enrofloxacin/ marbofloxcin

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AHL Newsletter 2024;26.

**Susceptible-dose dependent (SDD)** is a new interpretative category introduced by Clinical Laboratory Standard Institute (CLSI) guideline CLSI VET01S-ed7. SDD can be seen on AHL reports when minimal inhibitory concentration results are reported for antimicrobial susceptibilities. This category implies that susceptibility of an isolate depends on the dosage regimen. Currently, SDD category is only applicable in dogs when enrofloxacin or marbofloxacin is used in treatment of Enterobacterales (*E. coli, Klebsiella, Enterobacter, etc.)*, *Pseudomonas aeruginosa*, and *Staphylococcus* spp. Based on MIC values, the following dosage regimes are recommended:

| **MIC enrofloxacin** | **Category** | **Dosage regime** |
|---|---|---|
| ≤ 0.06 µg/ml | S | 5 mg/kg PO every 24 h or 2.5 mg/kg every 12 h |
| 0.12 µg/ml | SDD | 10 mg/kg PO every 24 h |
| 0.25 µg/ml | SDD | 20 mg/kg PO every 24 h |
| | | |
| **MIC marbofloxacin** | | |
| ≤ 0.12 µg/ml | S | 2.75 mg/kg PO every 24 h |
| 0.25 µg/ml | SDD | 5.5 mg/kg PO every 24 h |

**Reference**

1. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 7th ed. CLSI supplement VET01S. Clinical and Laboratory Standards Institute; 2024.

# Blastomycosis in a cat presenting as suspected mammary carcinoma

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AHL Newsletter 2024;28(3):26.

A 12-year-old female spayed cat was presented to her veterinarian for swelling and ulcerated lesions on her abdomen following a small laceration which occurred while in the garden. There were inflamed and excoriated growths noted on both sides of the caudal mammary gland that reduced in size with cleaning and treatment, but did not fully regress. Due to this lack of response, the top differential was inflamed mammary adenocarcinoma, and bilateral caudal mastectomy was performed. The tissue was submitted to the Animal Health Laboratory for histologic assessment.

On examination, the mammary tissue and associated lymph nodes were extensively effaced by coalescing nodules composed of abundant neutrophils, macrophages, and eosinophils surrounding large areas of necrosis that were in turn surrounded by lymphocytes, fibrous tissue, and plasma cells (**Fig 1).** There were frequent multinucleated giant cells in these areas. Within the foci of necrosis and inflammation there were numerous spherical, approximately 7 um to 15 um diameter organisms with a thick, double-contoured capsule which very rarely had evidence of broad-based budding (**Fig 2).** This histologic appearance is classic for the fungal infection blastomycosis.

Blastomycosis is a common fungal infection in dogs, and is rarely described in cats. The fungus is thermally dimorphic, growing as a mold in the cooler soil environment, and growing as a yeast in human or animal tissue. It is reported worldwide, and cases have been reported across Ontario. Endemic locations generally have moist, acidic soil which is the presumed reservoir for this agent. The noted laceration which occurred on the abdomen of the above-described cat while in the owner's garden is the most likely source of inoculation for the fungus. Gardening is a described risk factor for human blastomycosis cases due to disruption of the soil and possible inhalation of fungal spores or inoculation into the skin. Clinical presentations vary from respiratory or cutaneous infections to systemic disease. In the above-described case, only cutaneous lesions were noted, and no respiratory or systemic disease was described in the reported history. In previous studies of cats with blastomycosis, animals without respiratory signs often had radiographic evidence of respiratory disease. Therefore, systemic treatment is often started regardless of presentation.

Compared to blastomycosis, mammary neoplasia is very common in cats; it is the third most common tumor type in female cats after lymphoma and skin tumors. Mammary tumors are reported to represent 17 % of feline tumors, and the prevalence of malignancy is very high, with up to 90 % of these tumors reported to be malignant. Tissue necrosis and inflammation is common in these masses, which was mimicked by the fungal infection found in this case. While rare, blastomycosis should be considered as a differential diagnosis for masses in the mammary chain of cats. *AHL*

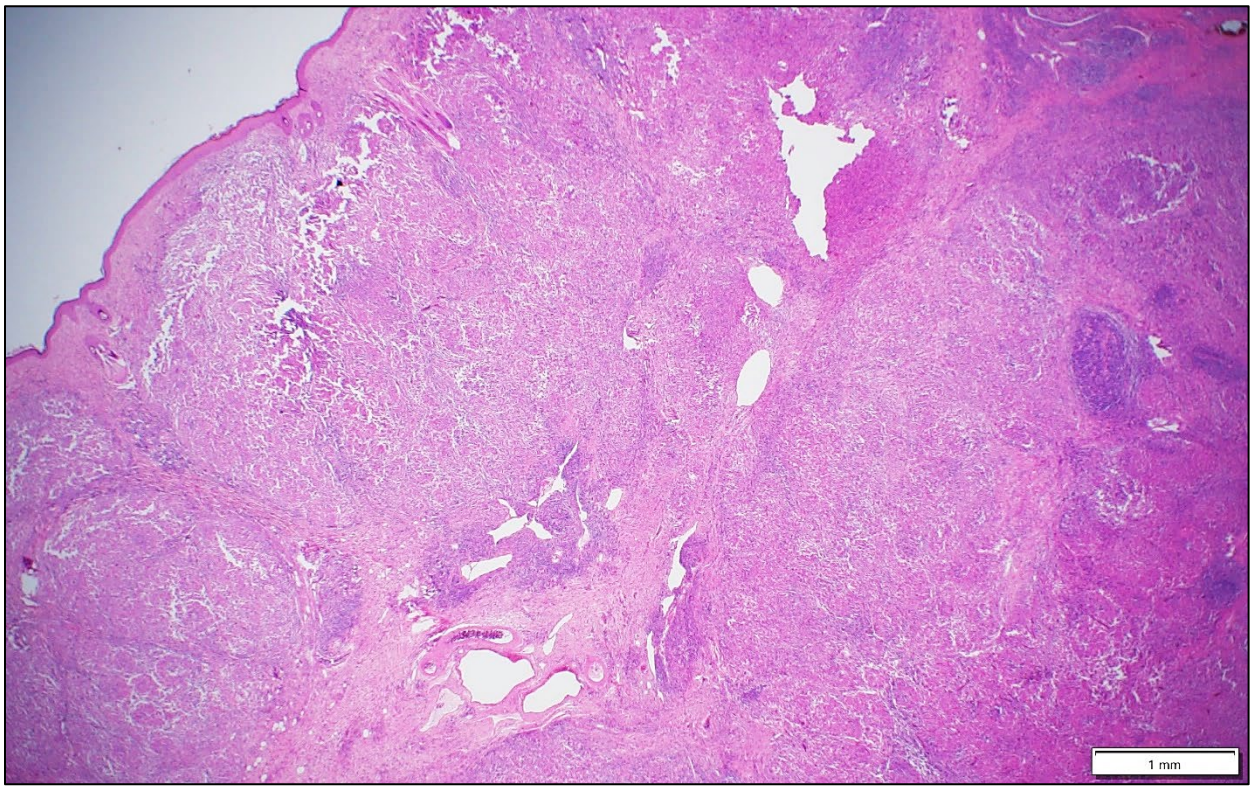

**Figure 1:** Low magnification histologic view of the mammary tissue showing extensive effacement by pyogranulomatous inflammation. The skin surface is at the upper left. H&E stain.

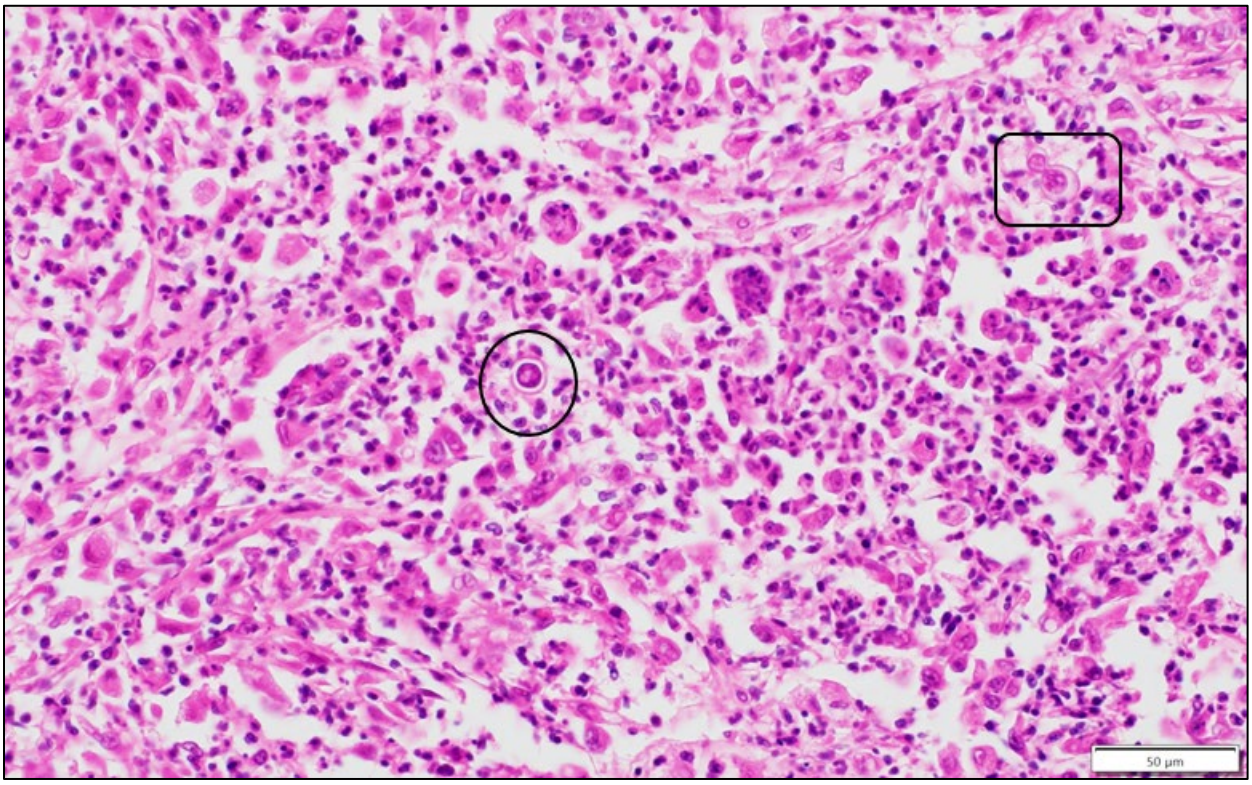


**Figure 2:** High magnification histologic view of the mammary tissue showing fungal organisms (inside black outlines) in a background of severe pyogranulomatous inflammation. One of the fungi is undergoing budding (black rectangle). H&E stain.

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