Laboratory Services Division

#### **Animal Health Laboratory**



**AHL** Newsletter

AHL Newsletter, Volume 28, Number 4

December 2024

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## AHL holiday hours 2024/2025

Except for Wed. Dec. 25 (closed – no service), AHL-Guelph is open every day from Mon. Dec. 23, 2024, until Wed Jan 1, 2025 with limited services. The University of Guelph is officially closed during this period.

Sat. Dec. 21	Guelph: specimen receiving, emergency mammalian postmortems, full			
	bacteriology set-up, as well as clinical pathology testing; Kemptville			
	closed			
Sun. Dec. 22	Guelph: specimen receiving, emergency mammalian postmortems;			
	Kemptville closed			
Mon. Dec. 23	All laboratory sections open with limited services (Guelph - 9:00 am to			
	5:00 pm; Kemptville 8:30-4:30 pm)			
Tues. Dec 24	All laboratory sections open with limited services (Guelph - 9:00 am to			
	5:00 pm; Kemptville 8:30-4:30 pm)			
Wed. Dec. 25	Guelph and Kemptville laboratories closed			
Thurs. Dec. 26	Guelph: specimen receiving, emergency mammalian postmortems;			
	Kemptville closed			
Fri. Dec. 27	All laboratory sections open with limited services (Guelph - 9:00 am to			
	5:00 pm; Kemptville 8:30-4:30 pm)			
Sat. Dec. 28	Guelph: specimen receiving, emergency mammalian postmortems, full			
	bacteriology set-up, as well as clinical pathology testing; Kemptville			
	closed			
Sun. Dec. 29	Guelph: specimen receiving, emergency mammalian postmortems;			
	Kemptville closed			
Mon. Dec. 30	All laboratory sections open with limited services (Guelph - 9:00 am to			
	5:00 pm; Kemptville 8:30-4:30 pm)			
Tues. Dec. 31	All laboratory sections open with limited services (Guelph - 9:00 am to			
	5:00 pm; Kemptville 8:30-4:30 pm)			
Wed. Jan. 1	Guelph: specimen receiving, emergency mammalian postmortems;			
	Kemptville closed			
Thurs. Jan. 2	Guelph and Kemptville - All laboratory sections open with full service			

Guelph drop box and fridges available 7AM to 10PM and Kemptville drop box and/or fridges are available 365/24/7 for specimen drop off.

For full details, please see our website - <u>www.ahl.uoguelph.ca</u>

Note: System generated invoices are scheduled for Dec 23 and 30, 2024.



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## Update from the Director



The view from the Director's office

AHL is fortunate to have an amazing complement of veterinarians on its staff. These pathologists and microbiologists, as well as our toxicologist, epidemiologist and client services veterinarian, all have advanced degrees and certifications in their area of specialization, providing AHL clients with the most up-to-date scientific expertise. Indeed, many of our clients cite the ability to easily contact an AHL veterinarian to discuss testing options or a challenging case as one of the main reasons for using our laboratory. Our veterinarians are multi-talented and are involved in many aspects of laboratory operations including supervision of staff, new test development, project management, and technological innovation. Because of our close relationship with the Ontario Veterinary College, many AHL veterinarians participate in DVM teaching sessions, in addition to serving on graduate student committees. Several veterinarians are also members of provincial, national or international professional organizations, generously providing their time and skills to advance their particular scientific objectives.

Therefore, it is very gratifying to have two AHL veterinarians - Dr. Josepha DeLay and Dr. Jan Shapiro - recognized for their exemplary contributions to the veterinary profession. Please check out the staff highlights section for complete details of their well-deserved awards.

From all of us at AHL, best wishes for the holidays, happiness, and peace in 2025.

#### Maria Spinato, Director

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

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## Update from Specimen Reception

Tim Pasma, Jennifer Zoethout Animal Health Laboratory, University of Guelph, Guelph, ON.

AHL Newsletter 2024;28(4):4.

#### **Cold weather shipping**

As winter approaches and the temperature become colder, please remember to package submissions to prevent freezing of samples. Samples such as EDTA tubes can be packaged with a room temperature ice pack to help prevent freezing. To prevent formalin from freezing, add 1mL of ethanol per 10mL of formalin.

#### Handling charge for coolers

Purolator has informed us they will be adding a handling charge for plastic or styrofoam coolers effective April 1, 2025. This charge will be added as these types of coolers cannot be processed on their automated lines and need to be handled manually. To avoid the handling charge, AHL recommends that coolers be packaged inside a cardboard box. The handling charge will be billed to the client by AHL.

#### Shipment of multiple packages

Please note that we cover the cost of shipping one package per day. If we receive more than one package shipped from a client on the same day, our policy is to bill back this cost to the client (at \$15.00 per shipment). The exception is for shipments of cytology slides and fixed tissues which must be sent in separate shipments to prevent damage to slides by formalin fumes.

## OAHN Update – December 2024



Mike Deane, Tanya Rossi Animal Health Laboratory, University of Guelph, Guelph, ON.

This fall, the Ontario Animal Health Network has continued to create new resources and reports for veterinarians, animal owners, and industry. The OAHN Companion Animal Network has released its 2024 Public Health Update, as well as a Podcast on FIP Treatments and where to find them. Additionally, many of the networks have created species-specific reports, which you will find below.

#### **OAHN Public Health Update 2024**

The Ontario Animal Health Network (OAHN) was created to achieve coordinated preparedness, early detection, and response to animal disease in Ontario. OAHN is a "network of networks" with individual networks for different species/sectors, each of which involves collaboration among veterinarians, animal owners and stakeholders in the field with laboratory, academic and government experts. This annual update is for public health professionals in Ontario, to highlight pertinent topics from the last 12 months from the OAHN companion animal and other species networks, and to help strengthen the link and communication between animal health and public health networks. Topics for 2024 include: Monkeypox in animals, H5N1 influenza, *Chlamydia caviae* in guinea pigs, updated Lyme disease infographic, acute dog diarrhea, raw meat-based diets infosheet, antimicrobials in dogs and cats, and much more. Access it here: https://www.oahn.ca/resources/ontario-animal-health-network-oahn-public-health-update-2024/

#### New Podcast: Feline Infectious Peritonitis (FIP) Treatments and Where to Find Them

Feline infectious peritonitis (FIP) occurs as a result of a viral mutation in cats infected with feline coronavirus. For decades, FIP has been a devastating condition in cats with an extremely high (95%) mortality rate and no reliable treatment options. A few years ago it was discovered that the antiviral drugs remdesivir and GS-441524 are highly effective for treating FIP, but these drugs are very expensive and there was no legal means of accessing them in Canada or the US – until now! As of 2024, Canadian veterinarians can access these life-saving drugs through an Emergency Drug Release (EDR). As these drugs and the process to access them are new to many Ontario veterinarians, the Ontario Animal Health Network is thrilled to have Dr. Kelly St. Denis walk us through some of the ins and outs of these new treatment options. Check out the information here: <a href="https://oahn.podbean.com/e/feline-infectious-peritonitis-fip-treatments-and-where-to-find-them/">https://oahn.podbean.com/e/feline-infectious-peritonitis-fip-treatments-and-where-to-find-them/</a>

#### **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 <u>OAHN.ca</u> and navigate to the species in which you are interested.

- OAHN fall survey and lab data: Key results
- Rabies update
- FIP update and NEW! podcast
- Changes to US dog import rules, AGAIN
- Mpox in animals: Good news, bad news
- Stay alert: H5N1 flu, cows, cats, mice
- Not-so-tropical mite
- Update: Lyme disease infographic

Equine Network - https://www.oahn.ca/reports/equine-veterinary-report-q2-2024/

- Strangles and EHV-1 resources
- BITS 'N SNIPS (or "things we talked about on the network call")
- EMPF
- Pasture-associated laminitis
- Network member reports
- Syndromic and lab surveillance dashboard
- Equine research
- ResearchONequine

## Bovine Network - <u>https://www.oahn.ca/reports/oahn-bovine-expert-network-quarterly-veterinary-report-q2-2024/</u>

- Q2 Bovine data from the Animal Health Laboratory
- Update on influenza A(H5N1) in U.S. dairy cattle
- Outbreak of Histophilus somni-associated bronchopneumonia at a calf rearing facility
- Seeking new network member
- Detections of *Cryptosprodium parvum* in Ontario

Swine Network - https://www.oahn.ca/reports/swine-veterinary-report-q2-2024/

- Novel influenza A: H3N2 cluster 2010.1 update
- Highly pathogenic avian influenza (H5N1) in dairy cattle in the U.S.A.
- Porcine epidemic diarrhea (PEDV)/Porcine deltacoronavirus (PDCoV)
- Senecavirus A (SVA)- EQSP Quebec update
- OAHN Swine project update- Porcine haemagglutinating encephalomyelitis virus (PHEV)
- OAHN veterinary clinical impression survey: Veterinary comments
- Laboratory diagnostic reports
- Ontario slaughter statistics
- International disease topics of interest summary

## Poultry Network - <u>https://www.oahn.ca/reports/oahn-poultry-expert-network-quarterly-veterinary-report-q3-2024/</u>

- Gangrenous dermatitis in commercial poultry
- Poultry veterinary survey highlights Q3 2024
- Research

## Staff highlights



Dr. Josepha DeLay is the recipient of the 2024 UGFA (University of Guelph Faculty Association) Veterinary Excellence Award. This award recognizes a significant contribution toward excellence in professional practice to the community. Congratulations Josepha!



Dr. Jan Shapiro (ret.) is the recipient of the 2024 CCVA (Central Canada Veterinary Association) lifetime membership award for all her contributions and involvement with the CCVA over many years. Congratulations Jan!

## Salmonella spp. serotyping available at AHL

#### Durda Slavic and Sarah Lippert

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

AHL Newsletter 2024;28(4):8.

The bacteriology section at AHL has validated the use of the Check&Trace *Salmonella* serotyping method (CTS). It is a PCR based method that can be used to differentiate more than 50 different *Salmonella* serotypes frequently isolated from poultry environmental samples, as well as from clinical samples in all animal species (**Table 1**).

AHL forwards *Salmonella* spp. to the Public Health Agency of Canada (PHAC) for serotyping. Frequently, this testing takes 3-4 weeks, and may take longer if environmental *Salmonella* spp. are submitted. When a faster turn-around-time (TAT) for serotyping is needed to confirm/exclude any of the serotypes from Table 1, the CTS test can now be requested. This test can only be done on pure bacterial cultures of isolates already identified as *Salmonella* spp. The price per isolate is \$75 and the test code is 'bssero'. If culture and/or bacterial identification are required prior to serotyping, extra charges will apply. For additional information please contact ahlbact@uoguelph.ca

S. Abaetetuba	S. Hadar	S. Panama
S. Agona	S. Havana	S. Paratyphi B (Java)
S. Alachua	S. Heidelberg	S. Poona
S. Albany	S. Idikan	S. Reading
S. Anatum	S. Infantis	S. Rissen
S. Bovismorbificans	S. Javiana	S. Saintpaul
S. Braenderup	S. Kentucky	S. Sandiego
S. Brandenburg	S. Livingstone	S. Schwarzengrund
S. Bredeney	S. London	S. Senftenberg
S. Cerro	S. Mbandaka	S. Stanley
S. Choleraesuis	S. Minnesota	S. Tennessee
<i>S</i> . Corvallis	S. Molade	S. Thompson
S. Cubana	S. Montevideo	S. Typhimurium
S. Derby	S. Muenchen	S. Typhimurium monophasic
		(1,4,[5],12:i:-)
S. Dublin	S. Muenster	S. Uganda
S. Enteritidis	S. Newport	S. Virchow
S. Gallinarum	S. Ohio	S. Worthington
S. Pullorum	S. Oranienburg	<i>S</i> . Yoruba
S. Give	S. Orion	Salmonella 4,[5],12:d:-
S. Goldcoast	S. Ouakam	

Table 1. Salmonella serotypes detected by CTS.

## RUMINANTS

## Pasteurella multocida meningoencephalitis in a heifer

Emily Brouwer

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

#### AHL Newsletter 2024;28(4):9.

In early September, a 4-month-old Holstein heifer presented to the referring veterinarian for euthanasia after suddenly developing neurological signs. This heifer had been examined and treated with meloxicam and florfenicol two days previously for suspected pneumonia, with no reported improvement. The morning of euthanasia, she was found down in lateral recumbency with opisthotonus. Her body temperature was 39°C. She was unable to maintain a sternal position, and it was noted that she had developed a mild ping over the right lateral abdomen in the region of the last ribs and the paralumbar fossa. She was euthanized and submitted to the AHL for postmortem examination. One other heifer in the same pen had died the day previously, and no postmortem examination was completed on her.

On external examination, the heifer was noted to be in good body condition and well hydrated. Internally, concentrated around the cerebellum and the ventral brainstem beneath the dura mater were sheets of yellow-tan, fibrinosuppurative exudate (**Fig. 1**). The lateral ventricles contained cloudy tan cerebrospinal fluid with flecks of fibrin. The exudate expanded the subdural space along the length of the spinal cord to the level of the lumbar spinal segments. The meningeal vessels were congested, and the leptomeninges were diffusely cloudy. Similar fibrinosuppurative exudate was present in the frontal sinuses. The tympanic bullae were clear.



**Figure 1:** Brain with severe fibrinosuppurative exudate overlying the cerebellum (1A) and brainstem (1B).

Approximately 50 % of the right cranioventral lung was consolidated, dark pink-red and very firm. On cut section, there was moderate bronchiectasis and peribronchial fibrosis, and airway lumens contained purulent exudate. There were occasional pinpoint-2 mm diameter white nodules in the pulmonary parenchyma in the affected region. Few dense fibrous adhesions were identified between the pericardium and epicardial surface.

Microscopic examination confirmed the gross diagnosis of severe meningoencephalitis and myelitis, typical of a bacterial etiology. Bacterial culture of the brain isolated 2+ *Pasteurella multocida* in pure culture. Bacterial culture of the lungs isolated 2+ *P. multocida*, as well as 2+ *Histophilus somni*. In this case, it is possible that prior *H. somni* bacteremia led to CNS vascular damage which allowed for hematogenous spread of *P. multocida* to the brain and spinal cord. The lesions in the brain were not typical of infectious thromboembolic meningoencephalitis, but there was significant vascular injury noted in several sections of the spinal cord. Other mechanisms of direct invasion could be through the ears (though no lesions were noted) or through dehorning wounds.

The pulmonary lesions were chronic and typical of a bacterial etiology as well. The typical caseonecrotic lesions of *Mycoplasma bovis* were not present in these sections, but the PCR was positive for *M. bovis* with a cycle threshold of 25.48, and thus this organism was considered a contributor to the respiratory disease. *Mycoplasma bovis* has been reported in association with fibrinous meningitis in cattle as well, presumably related to otitis media, which was not present in this case.

Bacterial meningitis in ruminants is typically described in the context of neonates developing bacterial meningoencephalitis from bacteremia secondary to omphalitis or severe enteritis, often as a sequel to inadequate passive transfer of colostral antibodies. In addition, direct extension via otitis externa, skull fractures or sinusitis have been described. In cattle, most bacteria identified in meningitis cases are gram negative, and *E. coli* is significantly over-represented. This case involves an unusual presentation of bacterial meningitis in a young heifer, with various potential pathogeneses.

#### Reference

1. Fecteau G, et al. Bacterial meningitis and encephalitis in ruminants. Veterinary Clinics: Food Animal Practice 2004;20(2):363-377.

## Juvenile lymphoma in a calf

Lisa Gordon

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

AHL Newsletter 2024;28(4):10.

A three-month-old Holstein heifer calf presented for generalized skin masses and increased respiratory effort of a few days' duration. On examination, the calf was tachypneic and tachycardic with a temperature of 40.2°C, and harsh lung sounds on thoracic auscultation. Peripheral lymph nodes were markedly enlarged (**Fig. 1**). In-house CBC showed markedly elevated lymphocytes, and euthanasia was performed due to poor prognosis. Postmortem examination revealed enlargement of internal lymph nodes and the formation of adhesions between abdominal viscera. A comprehensive suite of tissues was submitted for histopathology at the Animal Health Laboratory. The eighteen examined lymph node samples were effaced by monomorphic sheets of large neoplastic lymphocytes. Neoplastic lymphocytes were disseminated throughout the viscera, and were present within the spleen, liver, kidney, lung, heart, uterus, ovary, intestines, bladder, and abomasum (**Fig. 2**). The calf was diagnosed with disseminated large cell lymphoma, characteristic of juvenile lymphoma, also known as calf lymphoma.

Bovine lymphoma can be categorized based on factors such as age of onset, affected tissues, association with bovine leukemia virus (BLV), and the frequency of occurrence (sporadic vs. endemic/enzootic within a population). Juvenile lymphoma is a rare, sporadic form of bovine lymphoma that typically affects cattle younger than 6 months of age, usually between 3 to 6 months, although cases have been

reported in cattle ranging from 1 month to 3 years old. This form of lymphoma is considered spontaneous and is not linked to enzootic bovine leukosis (EBL) or BLV infection. Other types of sporadic lymphoma in cattle include thymic lymphoma, which primarily affects cattle between 6 months and 2 years, and cutaneous lymphoma, which generally affects cattle between 1 to 3 years of age. These forms of lymphoma are all non-infectious and non-contagious, and typically only individual animals within a population are affected. On the other hand, enzootic bovine leucosis is not sporadic, is associated with BLV infection, and generally affects cattle greater than 2 years of age. While EBL is characterized by a B-cell lymphoma, the sporadic lymphomas (juvenile, thymic and cutaneous) can originate from either B-or T-cell populations.

Clinically, young calves with juvenile lymphoma often present with generalized lymphadenopathy, while older calves may exhibit weakness, weight loss, and depression. Enlarged lymph nodes are typically smooth, firm, and non-painful on palpation. Additional clinical signs may include dyspnea, tachypnea, harsh lung sounds, tachycardia, and pallor. Some calves may also have fever, ruminal tympany, hepatomegaly, ataxia, and diarrhea. A complete blood count often reveals anemia and leukocytosis due to lymphocytosis. Bone marrow examination shows an increased myeloid-to-erythroid ratio and neoplastic lymphocyte proliferation. Neoplastic lymphocytes are found in lymph nodes and in various visceral organs, including the liver, spleen, pancreas, heart, uterus, and occasionally the thymus. However, the thymus is less frequently involved and less infiltrated by neoplastic lymphocytes compared to primary thymic lymphomas. The prognosis for juvenile lymphoma is grave, with affected calves typically succumbing to the disease within 2 to 8 weeks of the onset of clinical signs.



Figure 1. Markedly enlarged prescapular lymph node. Photo credit: Dr. Meggy Chan.



**Figure 2.** Histologic sections of tissues infiltrated by neoplastic lymphocytes. H&E stain. 2A. Lymph node. Neoplastic lymphocytes efface the normal architecture (asterisk). 2B. Liver. Subcapsular (thin arrow) and periportal (thick arrow) neoplastic lymphocytes. 2C. Uterus. Perivascular neoplastic lymphocytes (thin arrow). 2D. Ovary. Stromal neoplastic lymphocytes (thin arrow).

#### References

1. Angelos, JA. Proliferative disorders of the myeloid and lymphoid systems. In: Large Animal Internal Medicine. 6<sup>th</sup> ed. Smith BP, Van Metre DC and Pusterla N, eds. Elsevier, 2020;1180-1181.

2 Harbo SJ, et al. Characterization of lymphocyte populations by flow cytometry in a calf with sporadic juvenile lymphoma. Vet Clin Path 2004;33(3):163–167.

## SWINE

# *Brachyspira murdochii*-associated colitis in grower pigs: Guilt by association or innocent bystander?

Josepha DeLay, Clint Lichty

Animal Health Laboratory, University of Guelph, Guelph, ON (DeLay), South West Vets, Stratford, ON (Lichty)

AHL Newsletter 2024;28(4):13.

Grower pigs from a 1200 sow multi-site farrow-to-finish operation were examined due to persistent diarrhea involving 20% of the group. Nursery and grower pigs from the herd were housed in the same continuous-flow barn. Occasional diarrhea was noted in the nursery group, but became a more significant issue at the grower stage. Although some fading pigs were present, pigs with diarrhea were often in good body condition. Mortality was not increased.

Enteritis due to *Lawsonia intracellularis* was identified in nursery pigs and was considered the likely cause of ongoing diarrhea in growers as well. However, lack of response to treatment in the grower pigs prompted additional diagnostic testing. On-farm necropsy of euthanized pigs with clinical signs identified gross lesions of ulcerative colitis. Histologic lesions confirmed erosive to ulcerative colitis, and *L. intracellularis* was detected in feces or colon from multiple pigs by PCR (Ct 27-37); however, typical lesions of proliferative enteritis were not present. *Salmonella* spp. was not isolated from colon of multiple pigs, and *Trichuris vulpis* (whipworm) eggs were not detected in feces by fecal flotation.

*Brachyspira hampsonii* (Ct 37) was detected by PCR in the colon from 1 of 3 pigs examined, but the organism was not identified in other animals subsequently tested. PCR identified *Brachyspira murdochii* (Ct 22-29) in the colon from 3 of 4 additional pigs.

Erosive to ulcerative colitis was confirmed as a common lesion in affected grower pigs in this herd, but the definitive cause remains elusive. Contribution of *Brachyspira* spp., especially *B. murdochii*, to diarrhea in these pigs is suspected. Rather than simple and direct disease causation by this organism, diarrhea in this case is likely due to colonic dysbacteriosis (disruption and imbalance in the normal microbiota), potentially influenced by a number of factors. Importantly, infection of swine with any species of *Brachyspira* spp. does not necessarily cause disease, and the composition of the colonic microbial community influences disease expression in the form of diarrhea. In this case, pathogen build-up in the grower rooms is a suspected factor in the persistence of clinical diarrhea and was likely influenced by continuous flow and limited washing of grower rooms, and direct connection of nursery and grower rooms in the same barn. Subclinical infection with *L. intracellularis* may also be contributing to disruption of the normal colonic microbial community. Diet and the *Brachyspira* spp. profile in colon in other age groups in the herd, mainly sows, may have also influenced the grower situation. The pathogenic species *B. hampsonii* was confirmed in feces from only 1 pig and with a low pathogen load, suggesting that this finding may also be a manifestation of colonic dysbacteriosis in the group.

*B.murdochii* is considered an opportunistic pathogen in the colon of pigs. A report from Western Canada has identified a recent increase in the proportion of *B. murdochii*-positive clinical cases, suggesting that the organism may have an increasing role in swine colitis. Other investigations have also indicated that in some circumstances, *B. murdochii* is of low pathogenicity and can cause colitis in pigs. In the current case, *B. murdochii* was the only potential pathogen identified in multiple pigs with colitis. The organism may have contributed to colitis in these animals, but a more accurate interpretation may be that this finding demonstrates a detrimental shift in colonic

flora.

#### References

1. Costa MO, et al. Subclinical colitis associated with moderately hemolytic *Brachyspira* strains. J Swine Health Prod 2019;27(4):196-209.

2. Hampson DJ, Burrough ER. Swine dysentery and brachyspiral colitis. In: Diseases of Swine, 11<sup>th</sup> ed. Zimmerman JJ et al, eds. Wiley Blackwell, 2019:951-970.

3. Jensen TK, Christensen AS, Boye M. Brachyspira murdochii colitis in pigs. Vet Pathol 2010;47(2):334-338.

## AVIAN/FUR/EXOTIC

## Systemic myeloproliferative disease in a Silkie chicken

Andrew Brooks

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

#### AHL Newsletter 2024;28(4):15.

In the fall of 2024, a 1.5-year-old Silkie chicken was submitted to the AHL for postmortem examination. Clinically, the hen was keeping distant from the other birds, was not walking normally, and could not stand straight. The feces from the chicken were dark and had an abnormal odor. The bird had a previous history of respiratory signs.

At postmortem, the body cavity of the chicken contained hundreds of small, tan to white, miliary nodules throughout the mesentery and serosal surfaces. The kidneys were diffusely discolored white-tan and were enlarged. The liver also contained white-tan foci that appeared to accentuate the portal architecture. The ovary contained regressing follicles as well as multiple masses. The spleen was enlarged, had a firm texture and was diffusely pink on cut section. Histologically, the main finding was a myeloid cell population that had infiltrated several organs (**Fig. 1**). The myeloid cells were immature and exhibited a high nuclear:cytoplasmic (N:C) ratio and eosinophilic cytoplasmic granules. There were approximately 1-3 mitotic figures per 400x field. The myeloid proliferation affected the thymus, heart, lung, kidney, liver, spleen, ovary, oviduct and serosal surfaces of the intestinal tract, consistent with a diagnosis of **systemic myeloproliferative disease**.

This case of myeloproliferative disease is suspected to be a form of avian leukosis associated with the avian leukosis sarcoma group of retroviruses (ALSV), particularly myelocytomatosis associated with ALSV subgroup J (1). Further testing on this chicken was not performed, however, infection with ALSV may be detected by PCR or antigen ELISA. *AHL* 



**Figure 1.** Myeloproliferative disease in a Silkie chicken characterized by diffuse infiltration (\*) of kidney (A) and liver (B) by immature myeloid cells (inset). H&E stain.

#### Reference

1. Zavala G. Avian leukosis. In: Brugère-Picoux J, et al. Manual of Poultry Diseases, English edition. AFAS;2015:227-236.

## Avian adenoviral (FAdV-1) ventriculitis in laying hens

Emily Rätsep, Emily Martin, Davor Ojkic, Elizabeth Black

Animal Health Laboratory, University of Guelph, Guelph, ON (Martin, Ojkic), Kemptville, ON (Rätsep); Elfrida Poultry, Caledonia, ON (Black)

AHL Newsletter 2024;28(4):16.

In the late summer of this year, there was an increase in mortality since housing of commercial brown laying hens. The flock was placed at 20.5 weeks, with 0.66% mortality over the ensuing two weeks. Necropsies conducted at 23 weeks of age revealed the majority of birds contained melenic content in the crops, proventriculi and ventriculi. Ulceration and erosions were also observed grossly in the ventriculi. Tissues submitted for histologic examination confirmed an ulcerative and erosive ventriculitis with patchy areas of necrosis (**Fig. 1**). Within these necrotic regions, there were large, basophilic intranuclear inclusions within the remaining viable epithelial cells. Fowl adenovirus (FAdV) PCR on ventriculus was positive. Hexon-gene sequencing was carried out to determine the FAdV strain, and it was a 100% match to FAdV-1 (CELO).

While fowl adenoviruses are widespread and endemic worldwide, in Ontario they are most commonly associated with necrotizing hepatitis (inclusion body hepatitis). However, since the early 1990s, a FAdV-associated ventriculitis with intranuclear inclusions has been reported in both domestic chickens (broilers and layers) and captive bobwhite quail in multiple countries, including: Japan, Poland, Italy, Germany, Korea, the United Kingdom, the United States of America and Morocco. In each of these cases, reduced

weight gain and increased mortality were commonly noted, with grossly apparent melenic crop and gizzard content and proventricular/ventricular ulceration observed on necropsy. In these cases, histological findings confirmed the ulcerative and necrotizing ventriculitis, and large intranuclear inclusion bodies were often observed in mucosal epithelial cells. In all reported cases, the results of ancillary testing showed involvement of FAdV-1. While other FAdV strains were concurrently recovered in some cases, notably FAdV-8a, the few subsequently performed in-vivo trials noted that infection with similar gross and histological findings could be induced in birds with FAdV-1. Some reports indicate that vaccination against FAdV-1 provided protection in young broiler birds.

While these outbreaks noted increased mortality associated with FAdV-1 infection, outbreaks appeared to be sporadic and affected a relatively small proportion of the population. In each case, these outbreaks were described as occurring randomly over a period of years within the affected country, and marked sustained outbreaks were not reported.

Therefore, while ulcerative and necrotizing ventriculitis associated with FAdV-1 infection is only sporadically reported, it should be kept in mind as a differential diagnosis for increased mortality in laying hens or broilers with hemorrhagic/melenic crop and gizzard content. To our knowledge, this appears to be the first report of FAdV-1-associated ulcerative ventriculitis in laying hens in Ontario, Canada.



**Figure 1.** Ulcerative and necrotizing ventriculus with intranuclear inclusion bodies in a laying hen. There is ulceration and loss of the superficial mucosa, and remaining epithelial cells contain large basophilic intranuclear inclusions (arrows). H&E stain, 20x.

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## AHL assists in bear poaching investigation

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In Ontario, it is illegal to possess the gall bladder of a black bear that has been separated from the carcass, including from bears that have been otherwise lawfully harvested. The demand for bear gall bladders is due to perceived medicinal benefits and use in traditional Chinese medicine, though the substantial demand is often linked to poaching, international smuggling, black market trade, and organized crime.

In late 2023, pathologists from the Animal Health Laboratory and the Canadian Wildlife Health Cooperative assisted in an investigation surrounding the possession of three dried gall bladders from American Black Bears that were discovered in the possession of an angler in Gravenhurst, Ontario. The defendant claimed that the gall bladders were from pigs, and feigned ignorance to the laws surrounding the possession of gall bladders from bears despite being a licensed hunter.

The gall bladders were DNA tested at Trent University and confirmed to be from American black bears. Canadian Wildlife Health Cooperative (CWHC) pathologist Dr. Brian Stevens and AHL pathologist Dr. Emily Brouwer were able to assist the investigation with photographs of bear and porcine livers and gall bladders (**Figs. 1 & 2**), literature review, and professional opinion.

The combined efforts of the Ministry of Natural Resources Intelligence and Investigations Services Unit, the Forensic Center at Trent University, and veterinary pathologists at CWHC and AHL led to a rare conviction under section 50 of the *Fish and Wildlife Conservation Act*, ultimately leading to \$6500 in fines. *AHL* 

https://news.ontario.ca/en/bulletin/1004970/ontario-protects-black-bear-against-illegal-hunting-practices https://www.ontario.ca/laws/regulation/980666





Figure 1. Ursine (bear) gall bladder (left).

Figure 2. Porcine (pig) gall bladder (right).

## HORSES

# Toxigenic *Corynebacterium diphtheriae* isolated from a donkey and the Public Health response

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AHL Newsletter 2024;28(4):20.

A skin lesion swab from a 35-year-old neutered male donkey was submitted for bacterial culture and susceptibility. The donkey had a 6-8 week history of ongoing skin lesions on all four limbs with purulent discharge and odour. On routine bacterial culture, large numbers of *Streptococcus equi* subsp. *zooepidemicus*, *Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* were recovered. Unlike *S. equi* subsp. *zooepidemicus* and *P. aeruginosa*, isolation of *C. diphtheriae* in this case was unexpected.

*C. diphtheriae* is a causative agent of diphtheria, a communicable disease in humans, and it is rarely isolated from animals. It produces a diphtheria toxin which is responsible for clinical disease, mainly respiratory and cutaneous forms. Isolation of toxin positive *C. diphtheriae* from humans must be reported to the local public health unit and to the Ministry of Health. The donkey's isolate was tested at AHL for the presence of diphtheria toxin gene by qPCR. After the toxin gene was detected, this finding was reported directly to Ministry of Health resulting in contact tracing and an extensive public health investigation. As not all qPCR toxin positive isolates are toxin producers, toxin production was confirmed by a modified Elek test at the National Microbiology Laboratory in Winnipeg. Following public health investigation, no human contacts were culture positive for *C. diphtheriae*. The other animals on the premises were not tested, and the source of infection for the donkey remains undetermined. The donkey was treated with antimicrobials primarily targeting *P. aeruginosa*, and was doing well at last check. This was the first isolation of *C. diphtheriae* from an animal recorded at the AHL.

In addition to *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* can carry the diphtheria toxin gene and cause diphtheria-like infections in humans and animals. Human cases of diphtheria-like disease caused by toxigenic *C. ulcerans* isolates have been increasing. While zoonotic infections were previously associated with livestock exposure and consumption of unpasteurized milk, recent reports of domestic pets being source of *C. ulcerans* infections are increasing, with cats, dogs, and horses being implicated. Animals carrying *C. ulcerans* are frequently asymptomatic, however, clinical infections are also reported. In contrast, toxin-producing isolates of *C. pseudotuberculosis* and clinical infections associated with them are extremely rare.

Both *C. ulcerans* and *C. pseudotuberculosis* (e.g., from caseous lymphadenitis in small ruminants) are routinely cultured at AHL, with *C. ulcerans* being less frequently recovered than *C. pseudotuberculosis*. No routine qPCR for detection of diphtheria toxin gene is being done on any of these isolates, as reporting of toxin positive *C. ulcerans* and/or *C. pseudotuberculosis* from animals is not mandatory. As a result, the prevalence of diphtheria toxin gene carrying *C. ulcerans* and *C. pseudotuberculosis* isolates among AHL submissions remains to be established.

Clinicians should be aware that *C. ulcerans* and *C. pseudotuberculosis* isolates may have a potential to carry and produce diphtheria toxin, and that toxin-producing isolates may have a significant public health impact.

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### Equine respiratory panel

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AHL is pleased to announce a new equine respiratory panel that be available on January 1,2025. This panel will make it easier for equine practitioners to select appropriate tests to confirm or rule out the most important and current respiratory pathogens of horses. The panel will include testing for:

- Influenza A virus by PCR
- Equine rhinovirus A and B by PCR
- Equine adenovirus 1 by PCR
- Equid herpesviruses 1, 2, 4 and 5 by PCR
- Aerobic bacterial culture, including 1 minimum inhibitory concentration (MIC) susceptibility

Any extra susceptibility testing requested on a bacterial isolate will be an additional charge.

Samples required for the test are a virus transport medium (VTM) swab **and** a bacterial swab **or** a piece of affected tissue. PCR tests can also be done on scrolls from formalin-fixed paraffin embedded (FFPE) blocks. Please contact us for any questions regarding this new equine testing panel.

### Polyneuritis equi in a Dutch Warmblood

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#### AHL Newsletter 2024;28(4):21.

Following the euthanasia of a 15-year-old Dutch Warmblood mare with a long history of progressively worsening loss of tail tone, urinary incontinence and passive defecation/fecal retention, the animal was submitted to the AHL for postmortem.

On gross examination, there was significant hind end muscle atrophy most visible at the base of the tail, as well as sabulous urolithiasis, most likely associated with the inability to fully express urine (**Fig. 1**). The sacrocaudalis dorsalis muscle and ventralis muscle of the tail were markedly atrophied and mottled pale brown/white to completely white (**Fig. 1a**). Coccygeal muscles and regions of the gluteal muscles

were similarly affected. The urinary bladder was filled with a 15 cm x 10 cm x 7 cm foul smelling, puttylike mass of sabulous light yellow crystalline sediment (**Fig. 1b**).

Microscopic lesions of the nervous system were largely restricted to the sacral spinal nerves (cauda equina), and included a severe chronic granulomatous and lymphocytic polyradiculoneuritis with marked axonal degeneration and epineurial fibrosis (**Figs. 2b, 2c**). Additional changes in other organs included marked muscle atrophy of the tail muscles (**Fig. 2a**), and evidence of a chronic cystitis.

Polyneuritis equi (cauda equina neuritis) is an uncommon sporadic disease in horses that can result in chronic clinical manifestations including sensory and motor deficits involving the tail and perineum, and urinary and fecal incontinence. This syndrome is of unknown etiology, but is considered to be an autoimmune or immune-mediated disorder following a viral infection. Clinically, animals exhibit a slowly progressive peripheral neurological disorder localized to the sacrococcygeal nerves (cauda equina), and it is most often observed in females. Formerly known as cauda equina neuritis in horses, the name of the disease was updated to polyneuritis equi based on descriptions in which the involvement of spinal nerves at various levels of the spine and cranial nerves were also reported. Clinical signs attributable to cranial nerve root involvement include facial paralysis, head tilt, and wasting of the masticatory muscles. In this case, no lesions were found in nerves distant to the sacral nerves.



Figure 1. Gross lesions of polyneuritis equi. 1a. A cross section of the proximal tail comparing an affected (left) and an unaffected (right) horse. There is marked muscle atrophy and pallor of the sacrocaudalis dorsalis muscle (SCD) and ventralis (SCV) muscles of the tail in the affected horse (left). 1b. Excessive accumulation of calcium carbonate sludge within the urinary bladder is characteristic of sabulous cystitis/urolithiasis which is often associated with urinary bladder paralysis or other physical or neurologic disorders hindering complete emptying.



**Figure 2.** Histologic lesions of polyneuritis equi. H&E stain. **2a.** Marked skeletal muscle atrophy of the SCV with small, individualized skeletal myocytes surrounded by fibrous and adipose tissues. 4x. **2b.** The epineurium of sacral spinal nerve bundles is markedly expanded and fused with adjacent nerve bundles. Sacral spinal nerves are variably effaced by a cellular infiltrate. 4x. **2c.** Marked infiltration and replacement of degenerate nervous tissue by lymphocytes (black arrow) and macrophages (blue arrow). Streams of expansile epineurial fibrous tissue are also present (green arrow). 20x.

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## **COMPANION ANIMALS**

# Rectoanal B-cell lymphoma in a dog: The diagnostic importance of IHC

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AHL Newsletter 2024;28(4):24.

A ten-year-old spayed female border collie dog presented to the referring veterinarian for rectal prolapse. After limited response to antibiotic and topical osmotic treatment, punch biopsies were taken from a palpably thickened and multifocally ulcerated area of mucosa near the mucocutaneous junction of the anus, and were submitted to the AHL for histopathology. Microscopically, the mucosa and submucosa were effaced by a round cell neoplasm that abutted and sometimes invaded into the mucosal epithelium (**Fig. 1**). Lymphoma was suspected based on the tumour morphology and the location of the neoplasm. Positive staining of the neoplastic round cells for PAX5, a B lymphocyte marker, confirmed the diagnosis of lymphoma and identified the neoplasm as a B-cell lymphoma.

Prolonged tenesmus is a common cause of rectal prolapse in dogs and cats and is most frequently seen in animals with high burdens of intestinal parasites. It can also occur in association with intestinal neoplasia, as in this case. Other underlying causes include intestinal foreign bodies, dystocia, urolithiasis, constipation, congenital defects and prostatic disease. In a small retrospective study of 11 dogs with rectal lymphoma, tenesmus was a common presenting complaint. All cases in this study were identified as B-cell in origin, and the prognosis was favourable with prolonged survival in dogs receiving chemotherapy.

Definitive histologic diagnosis of neoplasms can sometimes be difficult with hematoxylin and eosin staining alone, even with provision of relevant clinical histories and good gross descriptions. Immunohistochemical (IHC) staining uses antibodies to stain proteins expressed by neoplastic cells, and can be an invaluable tool for cell identification and tumour diagnosis. In cases of lymphoma, IHC is required for identification of B or T cell origin; it is not possible to differentiate between B and T lymphocytes with hematoxylin and eosin staining. Immunohistochemical staining for at least two proteins, a B cell protein and a T cell protein, is necessary and, in some cases, additional B cell markers are needed. In this case, the neoplastic cells expressed PAX5, the B cell protein, but not CD3, the T cell protein, providing important information for treatment (**Fig. 2**).



**Figure 1.** The neoplasm is composed of sheets of round cells with numerous mitotic figures (arrows). H&E stain.



**Figure 2.** Most neoplastic round cells have positive nuclear PAX5 staining (arrow, left image). Only a few scattered infiltrating non-neoplastic T lymphocytes have positive cytoplasmic CD3 staining (arrow, right image).

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### Sudden cardiac death due to caffeine toxicosis in a dog

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A 4-year-old Shetland sheepdog died suddenly 2 hours after an acute onset of vomiting. The family found some "mystery ground meat" surrounded by dead slugs in the backyard. Malicious poisoning was suspected, and the carcass was submitted to the Animal Health Centre in Abbotsford, BC, for postmortem examination. The dog weighed 14.2 kg and had abundant subcutaneous and visceral adipose tissue, with an estimated body condition scoring of 6/9. Gross examination revealed severe diffuse congestion of the liver and kidneys, acute pulmonary edema and congestion, and cyanosis of mucous membranes (**Fig. 1**), suggesting possible acute heart failure as the cause of death. Histologic findings were consistent with those of the gross examination. In addition, skeletal muscle myocytes occasionally had flocculated cytoplasm and contraction bands.



Figure 1. Postmortem examination. Severe congestion of the lungs and liver suggestive of acute heart failure.

Liver samples and the suspect meat were submitted to the AHL toxicology section for analysis. Upon examination, the mystery meat was noted to contain multiple small (1-2 mm) white particles/crystals (**Fig. 2A**). During the extraction for chromatography, an unusual pure white precipitate was noted at the bottom of the tube. These samples were forwarded to the AFL Microscopy laboratory, and the crystals in

the meat and white precipitate were identified as **pure caffeine** by Fourier Transform Infrared Spectroscopy (FTIR) (**Fig. 2B**). The liver and meat samples were negative for hundreds of targeted pesticides and drugs by GC-MS/MS and LC-MS/MS, however, caffeine was found in both samples with the open GC screen. A sub-sample of the meat was forwarded to an external laboratory for caffeine quantification and the concentration was 49,100 ug/g (approximately 5%).

The  $LD_{50}$  for caffeine in dogs is 140 mg/kg; therefore, a small 40 g piece of the meat patty would have been enough to deliver a lethal dose to a 14 kg dog, and even smaller doses have been reported to be toxic to dogs in other cases. These findings, along with identification of caffeine in the liver (confirming exposure), rapid clinical course and postmortem findings supported a diagnosis of caffeine toxicosis. The source was suspected to be crushed caffeine pills that are easily accessible and may contain up to 200 mg of caffeine per tablet.

Excessive caffeine can lead to severe cardiovascular problems, including tachycardia and arrhythmias, potentially causing sudden cardiac death. Gastrointestinal (vomiting and diarrhea) and nervous system (hyper-reactivity, ataxia, seizures) signs are also common. Caffeine has been used as an ecological alternative to control slugs, but it is typically applied in the form of coffee grounds to the soil, or caffeine tablets dissolved in water. In this case, addition of presumptive crushed caffeine tablets to ground meat was unusual and concerning for malicious poisoning.



**Figure 2.** Mystery meat found in backyard. 2A. Small white crystals in the meat were removed for analysis. 2B. Fourier Transform Infrared Spectroscopy (FTIR) spectrum showed an almost perfect match with the caffeine reference.

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## Tyzzer's disease in a kitten

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A young kitten became the focus of an animal welfare investigation due to a history of intractable diarrhea and emaciation. The owner had reported that they had owned the kitten for approximately two months, and in the two weeks prior to presentation the kitten had stopped eating and drinking. Apart from deworming one month previously, there was no reported medical treatment. On the morning of presentation to the veterinary clinic, the kitten was found to be moving erratically, gasping, and unable to stand. On antemortem physical examination, the kitten was pale, cold, thin, bradycardic and comatose. The kitten was euthanized and subsequently submitted for postmortem examination.



Figure 1. Emaciated and dehydrated kitten.

Figure 2. Perineal fecal soiling and dermatitis.

On gross postmortem examination, the kitten was emaciated and dehydrated (**Fig. 1**). Diarrheic feces soiled the perineum, tail and caudal thighs, and the underlying skin was hyperemic, swollen and coated in purulent exudate (**Fig. 2**). Internally, the gastrointestinal tract was empty, except for scant grey-brown liquid feces in the colon. The bone marrow was red and gelatinous, and internal fat stores were absent.

At the time of gross post mortem, pooled large intestine and small intestine were submitted for bacterial culture, which isolated 3+E. *coli* and 1+C. *perfringens*; these were interpreted as normal enteric flora. The *C. perfringens* enterotoxin ELISA was performed and enterotoxin was not detected. No *Yersinia* spp., *Campylobacter* spp., or *Salmonella* spp. were isolated. Liver was also cultured, and there was no bacterial growth. PCRs were also performed for *Giardia* spp., *Tritrichomonas foetus* and feline parvovirus, and all were negative.

Histologic examination was hampered by the degree of autolysis and freeze-thaw artifact, however, in sections of liver there were numerous, randomly distributed and variably sized foci of acute necrosis (**Fig. 3**). No significant enteric lesions were identified. Because the liver had previously been cultured with no bacterial growth, and subtle faint bacilli could be identified on routine staining, a Steiner stain was performed to identify argyrophilic bacteria. This stain identified large numbers of positive filamentous rod-shaped bacteria within and surrounding areas of acute hepatic necrosis, as expected with *Clostridium piliforme* infection (**Fig. 4**).



Figure 3. Liver, H&E stain, 10X. Multiple pale foci of acute hepatocellular necrosis (arrows).



**Figure 4.** Liver, Steiner silver stain, 40X. Numerous argyrophilic filamentous rod-shaped bacteria at the junction of viable and necrotic tissue.

This combination of characteristic hepatic lesions and positive identification of compatible intralesional bacteria is considered pathognomonic for Tyzzer's disease, confirming the diagnosis in this kitten. While not a common disease in cats, there are sporadic cases of *Clostridium piliforme* infection in kittens that can result in the classical triad of lesions including colitis, hepatitis and myocarditis. Perianal dermatitis with intraepithelial bacteria is also reported, but is not a common feature of the disease. The dermatitis is considered to be the result of direct contact with infected feces. While not identified in this case, likely due to the degree of autolysis, the expected histologic lesions in the gastrointestinal tract include neutrophilic crypt abscesses, crypt degeneration and loss, and ulcerative typhlocolitis and proctitis with mixed lymphoplasmacytic and neutrophilic infiltrates. Intraepithelial rods are often difficult to identify with routine staining, and typically require special silver impregnation stains.

*C. piliforme* is an obligate intracellular bacterium, which renders culture challenging. Isolating the bacteria in cell-free media is impossible, but the bacterium may grow in tissue culture under anaerobic conditions. Molecular techniques are the preferred method of confirming the diagnosis, particularly on antemortem examination. A PCR test can be performed on feces or affected tissues. The Steiner stain is typically more sensitive on formalin-fixed paraffin embedded tissues than the PCR for identifying the bacteria.

In cats, this infection appears to be more common in younger kittens, and is speculated to be related to impaired immune function. In cases of hepatitis or colitis in young kittens, particularly those that have been orphaned or have poor nutritional status, *C. piliforme* infection should remain a differential diagnosis.

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### C-reactive protein: A major acute phase protein in dogs

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#### AHL Newsletter 2024;28(4):30.

Acute-phase proteins are produced by the liver in response to tissue damage and play a role in the innate immune response. The acute phase protein profile differs from species to species, with some proteins exhibiting the characteristics of a major acute phase reactant in one species, yet only negligible increases within another.

C-reactive protein (CRP) is a major acute phase protein in dogs. Increased hepatic production begins within 4 hours of a stimulatory event, leading to increased serum concentrations reflective of both protein production and peripheral catabolism. CRP is present in negligible amounts in healthy animals, but can

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increase rapidly and dramatically (up to 100-fold) following an inciting event; the magnitude of this increase is dependent upon the nature of the insult. Stimulatory events can include inflammation, infection, immune-mediated disease, neoplasia and trauma. Concentrations decrease rapidly with resolution of the underlying stimulus.

CRP can complement CBC (complete blood count) and serum biochemistry findings. CRP will start to decrease earlier than neutrophil concentrations as inflammation or infection subsides in response to treatment, and can therefore be used to guide duration of therapy. CRP concentrations appear to be unaffected by hydrocortisone treatment in healthy dogs, thus CRP may be helpful in monitoring response to therapy in dogs receiving corticosteroids. Steroid therapy typically results in peripheral neutrophilia, thus reducing the clinical utility of the CBC. CRP has been shown to help differentiate bacterial versus other (e.g., eosinophilic, cardiogenic) causes of respiratory disease. Dogs with bacterial discospondylitis diagnosed by MRI exhibited markedly increased CRP concentrations, although pyrexia and leukocytosis were only variably present. Persistently increased concentrations of CRP in dogs with pancreatitis was associated with a poor clinical prognosis. CRP has been shown to be increased in dogs with mammary tumors, lymphoma, mast cell tumours, sarcomas, and metastatic neoplasia. However, the magnitude of CRP increase was not always correlated with tumour grade or burden. In dogs receiving therapy for lymphoma, remission status was best assessed by comparison to individual baseline CRP concentrations rather than to a CRP reference interval. It was suggested that the utility of CRP for the diagnosis and monitoring of malignancy may be enhanced by concurrent measurement of other markers of cellular proliferation.

The AHL Clinical Pathology laboratory now offers a **canine specific CRP assay (test code: crp)** which requires a minimum of **0.5 ml of serum**. A preliminary study within our lab utilizing 36 clinically well dogs resulted in CRP concentrations from 0 mg/L to 14 mg/L, similar to those reported by others. Clinical usage of CRP within our laboratory setting is relatively new; however, evaluation of this acute phase protein may be helpful in those instances where inflammation or infection are clinically suspected but changes in the CBC and serum biochemistry profiles are equivocal, and for monitoring response to therapy.

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