Laboratory Services Division
Animal Health Laboratory
University of Guelph
Changing Lives Improving Life

AHL Newsletter

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In this issue:

Dr. Brent Hoff is retiring!

After many years of dedicated service to the Animal Health Laboratory and before that to the Veterinary Laboratory Services Branch of OMAFRA, Dr. Hoff is retiring. A 1969 DVM graduate of the Ontario Veterinary College, Brent was the practice owner of the Taunton Road Animal Hospital in Oshawa for 8 years before returning to OVC to complete a DVSc degree in clinical pathology, followed by a Diploma in Toxicology. While working as our clinical pathologist and clinical toxicologist, Brent became well know to practitioners throughout Ontario and beyond.

We wish Brent a long and happy retirement - after he finishes providing part-time mentoring to his successors in the lab! *AHL*

Dr. Emmeline Tan joins the AHL

Dr. Tan joins us on a part-time appointment from August 1, 2013, until April 30, 2014, as a clinical pathologist and clinical toxicologist. A 2000 DVM graduate of the OVC, Emmeline was in small animal emergency practice for several years, before returning to OVC to earn a DVSc in clinical pathology in 2010. As well, she earned diplomate status in clinical pathology from the American College of Veterinary Pathologists in 2009.

We're delighted to have Dr. Tan join the AHL team! *AHL*

We've gone green!!

As of September, 2013, we have migrated to electronic publication of the AHL Newsletter. The AHL Newsletter has been available on-line for several years, and is easily accessible at http://guelphlabservices.com/AHL/Newsletters.aspx

Notification and Table of Contents of each newsletter will be sent to all addresses currently on our email distribution list - click on any topic of interest. **If your practice is on the list, but you would like to receive a personal notification, please send your request to: *holiver@uoguelph.ca***

We welcome your feedback.

How to "Contact us"

First choice: email - If clients contact the AHL by email, we have an exact record of what you've asked for, you aren't waiting on the phone or being redirected, you don't leave a voicemail for someone who is away, and your service experience will be optimal. The following group email addresses are monitored throughout the day by lab staff, and we do our very best to give you prompt attention.

General enquiries: ahlinfo@uoguelph.ca

Lab-specific enquiries – drop tests, add sequencing, etc.:

Bacteriology – bacteriology@uoguelph.ca
Clinical Pathology – clinpath@uoguelph.ca
Histotechnology - ahlhisto@uoguelph.ca
Molecular Biology - ahlmolecularbio@uoguelph.ca
Mycoplasmology - mycoplasmology@uoguelph.ca
Parasitology - ls.parasitology@uoguelph.ca
Virology - ahl.virology@uoguelph.ca

Second choice: telephone/voicemail - If you don't have our extension handy, the UofG central switchboard has a "speech attendant" system wherein you dial the main number 519-824-4120 and say the person's name to be connected. *AHL*

Maximizing the benefit of surgical biopsies

Andrew Brooks

The key to a successful biopsy is to carefully identify, harvest and preserve the most diagnostically relevant lesions. This article focuses mostly on skin and tumor biopsies but is applicable to a range of sample types.

A biopsy ideally provides a definitive diagnosis that guides case management. In situations where a definitive diagnosis is not obtained, histological examination of lesions can be useful for ranking a list of differentials, or may identify the likely pathogenesis (e.g., immune-mediated). Note that a biopsy alone is no substitute for a thorough clinical work-up.

A biopsy may be useful in many situations:

- To diagnose suspected neoplastic conditions.
- To confirm a diagnosis prior to selecting therapies which may be costly or potentially harmful (e.g., chemotherapy).
- To investigate unusual clinical presentations or persistent lesions (such as non-healing ulcers).
- To investigate conditions that appeared routine but have not responded to therapy.
- When differential diagnoses cannot be discriminated further by clinical examination or other less-invasive tests.
- When histopathology is necessary to diagnose the suspected disease (e.g., sebaceous adenitis).

It is most rewarding to biopsy early in the course of a disease when the primary lesions are acute and plentiful. This is especially true for skin conditions where chronic secondary lesions can mask the underlying problem. Interpretation of skin biopsies can be confounded by treatment effects. **It is recommended to biopsy before starting treatment and that anti-inflammatory treatments (glucocorticoids) are stopped 2-3 weeks prior to biopsy.** Secondary bacterial pyoderma complicates many dermatoses, and should be treated prior to biopsy.

Biopsy submissions should include a pertinent, concise (and legible!) history with key physical exam findings, description of the lesions, differential diagnoses, and the results of relevant lab tests or therapeutic trials. Describe the type and distribution of primary and secondary skin lesions (Table 1) and mention specific locations that may be diagnostically important such as nasal planum, paw pads and mucocutaneous junctions. **Clinical photographs are welcomed, and remember to include any specific questions that you want the pathologist to answer.**

Continued on p. 19

Table 1. Primary and secondary skin lesions

Primary lesions	macule, papule, pustule, vesicle/bulla, wheal, nodule/tumour, cyst
Secondary lesions	epidermal collarette, excoriation, scarring, erosion/ulcer, fissures, lichenification, callus
Lesions that may be primary or secondary	alopecia, scale, crust, follicular casts, comedo, pigment changes

AHL Newsletter
September, 2013 - Volume 17, Number 3

Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistants: **Helen Oliver, April Nejedly**
The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends.

Mailing address & contact information:
(**please return all undeliverable Canadian addresses to**:)
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
Email: holiver@uoguelph.ca
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Contributors to this issue:
From Laboratory Services / Animal Health Laboratory:
Andrew Brooks, DVM, PhD, Diplomate ACVP
Josepha DeLay, DVM, DVSc, Diplomate ACVP
Jim Fairles, DVM, MBA
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Emily Martin, DVM, MSc, Diplomate ACPV
Davor Ojkic, DVM, PhD
Kristiina Ruotsalo, DVM, DVSc, Diplomate ACVP
Nick Schrier, MSc
Durda Slavic, DVM, PhD

Other contributors:
Ann Godkin, DVM, DVSc, OMAF/MRA, Elora, ON
Ken Leslie, DVM, MSc; **David Kelton**, DVM, PhD; **Laura Pieper,** DMV, MSc; Dept Population Medicine, OVC
Angela Polleichtner, DMV; **Uwe Roesler,** Dipl. ECPHM, DVM; Free University of Berlin
David Ross, DVM, Berwick, Nova Scotia

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

Surgical biopsies continued from p. 19

Site selection is one of the most important factors that determines the usefulness of a biopsy. It is important to select multiple sites (i.e. 3-5 punch biopsies) that contain acute primary lesions as well as relevant secondary lesions. Secondary lesions that evolve from primary lesions may be diagnostic, whereas those that result from self-trauma are usually not. Selecting secondary lesions is aided by an understanding of the pathogenesis of the diseases on your differential list. For example, in pemphigus foliaceus, the pustules often transition to erosions and crusts as they rupture, and it is not uncommon to make this diagnosis based solely on the submitted crusts!

In **depigmenting** skin conditions, the most diagnostic lesions are often present at the site of active pigment loss (grey skin) where the disease process should be most pronounced. The cause of a cutaneous **ulcer** is often most evident at the ulcer margin. In cases of **alopecia**, select samples from the center of the most alopecic regions as well as junctions with normal skin. (Technical note: for suspected hair follicle diseases, *mark the direction of the hair coat on the biopsy by drawing a line with a fine-point permanent marker* – this allows the sample to be trimmed with optimal orientation of the hair follicles). With depigmenting and alopecic conditions, it can also be useful to include a region of normal skin for comparison. Remember to clearly label the different samples in separate jars. Other ways to distinguish multiple samples include sutures (good for tumors) and tissue cassettes (for very small samples).

Punch biopsies are effective for most skin conditions but an **elliptical excision** performed with a scalpel is preferred for fragile lesions, such as large pustules, diseases of the subcutaneous fat (panniculitis), tumors, and when attempting to sample the junction between abnormal and normal skin. Remember to not surgically prepare the biopsy site by scrubbing with antiseptics as this may remove diagnostic features such as crusts. Ensure that the primary lesions are centered in the specimen since punch biopsies are typically bisected down the center when sectioned at the laboratory (if you suspect that a lesion has been missed please contact the pathologist – it may be that additional sections are required).

Biopsies should be handled gently to minimize artifact and placed as soon as possible in fixative (10% neutral buffered formalin, with a minimum 10:1 volume ratio of formalin to tissue). Laser or cautery should not be used for skin biopsies. In the winter, freezing artifact may occur during transport. Freezing artifact may be avoided by ensuring tissues are fully fixed prior to shipping (usually by 24 hours), and fixed tissues can also be sent in a solution of 9 parts formalin to 1 part alcohol. To minimize shrinkage or curling of larger skin biopsies the tissue can be placed (fat side down) onto a wooden tongue depressor to stabilize the tissue during fixation.

For small tumors (i.e., < 1-2cm), submit the entire excisional biopsy for diagnosis and margin evaluation. Remember that formalin only penetrates approximately 1cm into tissue - to promote fixation of larger specimens, it is helpful to partially incise the tissue at 1cm intervals. For very large tumors (> 10 cm), representative samples of the mass and margins can be submitted for evaluation. For complex tumor samples, such as amputated limbs or whole spleens, please contact the laboratory in advance to discuss tissue sampling and shipping with a pathologist.

Evaluating completeness of excision is assisted by identifying relevant tumor margins with ink. This is particularly helpful for larger tumors that are often cut into smaller pieces for transport. Surgical ink is commercially available in various colors for identifying different margins of a resected tumor. To avoid the ink leaching onto other tissues, blot the margin with gauze to remove excess blood and liquid, apply the ink with a cotton swab, and allow the ink to dry for a few minutes prior to immersion in formalin.

Some key points to remember when submitting surgical biopsies:

- Select multiple representative samples that contain primary (diagnostic) lesions.
- Biopsy early, before non-specific secondary lesions predominate.
- Avoid confounding treatment effects, and treat secondary pyoderma first.
- Handle and preserve tissues properly.
- Include crusts.
- Provide a concise, relevant and legible history.
- Identify margins of interest with ink or sutures.

Feel welcome to discuss your case with the pathologist. *AHL*

References

Bettenay, SV, Hargis AM. Practical Veterinary Dermatopathology for the Small Animal Clinician. Jackson, WY: Teton NewMedia, 2006.

Miller WH, et al. "Chapter 2 Diagnostic Methods." Muller & Kirk's Small Animal Dermatology. 7th ed. St. Louis, MO: Elsevier/ Mosby, 2013.

AHL Lab Reports

RUMINANTS

New test for iodine in milk at the AHL

Nick Schrier

* Developed and validated for the determination of iodine in raw or processed cattle, goat, sheep, and water-buffalo milk.
* 5 mL of raw or processed milk is required, and ship samples on ice packs.
* Testing is performed once a week, and the fee is $30.00 per test

For more information, please contact Nick Schrier at nschrier@uoguelph.ca or 519 824-4120, ext. 57215. *AHL*

Confirmed case of bovine anaplasmosis in eastern Ontario

Kristiina Ruotsalo, Emmeline Tan

(Abstracted from the Ontario Ministry of Agriculture and Food (OMAF) disease advisory, with permission)

In late July, 2013, a blood smear collected by the herd veterinarian in the United Counties of Stormont, Dundas and Glengarry from a Holstein cow with decreased milk output looked suspicious for anaplasmosis on examination at the Animal Health Laboratory (Figure 1). CFIA conducted additional cELISA and PCR testing and confirmed *Anaplasma marginale* infection. The affected cow is under veterinary treatment and is reported to be recovering.

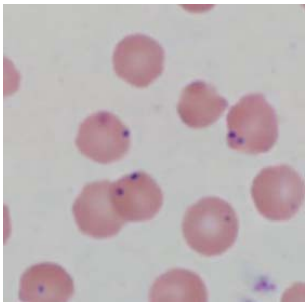

Figure1. *Anaplasma* organisms in bovine red cells

Anaplasmosis has only been diagnosed once previously in Ontario, in 1996. **Anaplasmosis is a federally reportable disease and a provincially (Ontario) immediately notifiable disease**. CFIA has placed the farm under quarantine, will be testing all cattle on the premises, and will be carrying out an epidemiological investigation to determine the source of the infection. All animals found to be infected will be culled according to CFIA protocol. Owners of all contiguous (within 2 km) herds, and all herds that have purchased animals from the affected farm, will receive a letter from the CFIA recommending they consider having their herds tested for anaplasmosis by their herd veterinarian..

Herd characteristics and cow-level factors associated with *Prototheca* mastitis on dairy farms in Ontario

Laura Pieper, Ann Godkin, Uwe Roesler, Angela Polleichtner, Durda Slavic, Ken Leslie, David Kelton

Prototheca spp. is an alga that causes incurable acute or chronic mastitis in dairy cows. The identification of cow and herd level risk factors for this unusual mastitis was the aim of the present case-control study. Aseptically collected composite milk samples from 2,428 milking cows in 23 case and 23 control herds were collected between January and May 2011, a questionnaire was administered to the producers, and cow level production and demographic data were gathered. In 58 of 64 isolates, *Prototheca spp.* and *Prototheca zopfii* genotypes were differentiated using MALDI-TOF MS. All those isolates were identified as *Prototheca zopfii* genotype 2. The mean within-herd prevalence for *Prototheca spp.* was 5.1% (range 0.0-12.5%). Case herds had a significantly lower herd-level prevalence of *Staphylococcus aureus* and a higher prevalence of yeasts than control herds. The final logistic regression model included intramammary injection of a non-intramammary drug (ImINID; OR = 127.6), the number of different injectable antibiotic products being used (NrInjectable; OR = 2.89), the use of any dry cow teat sealant (Teatsealant; OR = 35.5), and having treated 3 or more displaced abomasums in the last 12 month (≥3DA; OR = 41.1). The final logistic regression model for cow level risk factors included second or higher lactation (≥2nd lactation; OR = 4.40) and the logarithm of the lactation average somatic cell count (LASCC; OR = 2.99).

These results indicate that suppression of the innate udder flora through use of many different antibiotics, the introduction of *Prototheca spp.* through possibly unsanitary or repeated intramammary infusions, and impairment of the udder tissue through off-label use of injectable drugs in the udder might promote *Prototheca* udder infection.

(Published in Journal of Dairy Science 2012;95:5635-5644)

SWINE

Lawsonia intracellularis enteritis - still commonly seen in the lab

Murray Hazlett, Josepha DeLay

We love to show off good photos. Looking at the photos on this page, *Lawsonia intracellularis* enteritis is a good bet based on the gross lesions seen. The other major rule-out is salmonellosis.

Since the spring of 2007, pathologists at the AHL have diagnosed 173 cases of *L. intracellularis* enteritis. This does not include cases diagnosed without pathology (i.e., bacteriology smears or PCR testing only). Excluding one 4-week-old outlier (error on submission form?), the lowest age was 6 weeks, with a median age of 12 weeks. Some mature animals were also submitted.

The postmortem photos (Figures 1-3) are from 3 separate 10-week-old pigs from the same farm, and show the spectrum of lesions, some being more necrotic, some acute and hemorrhagic, and some primarily proliferative. Colitis is also present in some cases.

We have recently incorporated an IHC test for *Lawsonia* spp. (Figure 4) as well as PCR testing. *AHL*

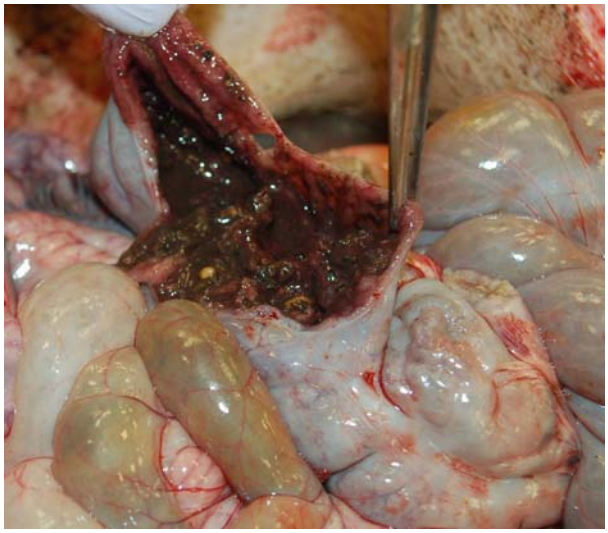

Figure 1. Severe hemorrhagic typhlitis (*Lawsonia*) - 10 week old pig.

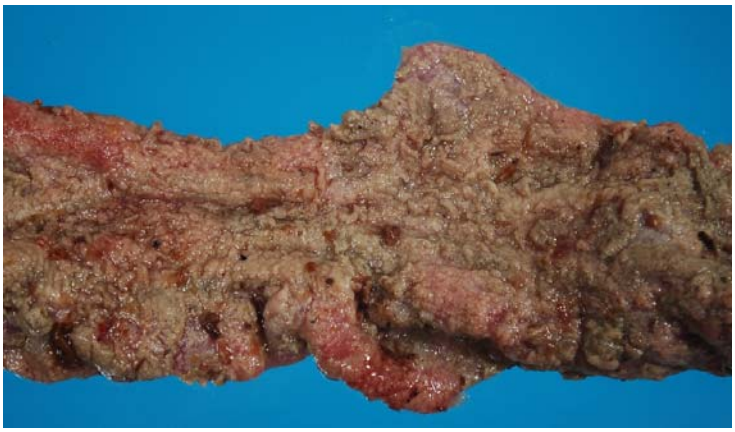

Figure 2. Necrotizing ileitis with proliferation from herdmate (*Lawsonia*).

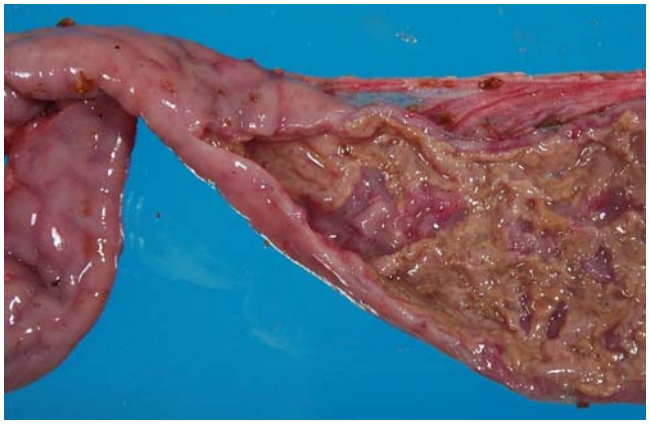

Figure 3. Mucosal hyperplasia and necrosis, proximal ileum/distal jejunum from herdmate (*Lawsonia*).

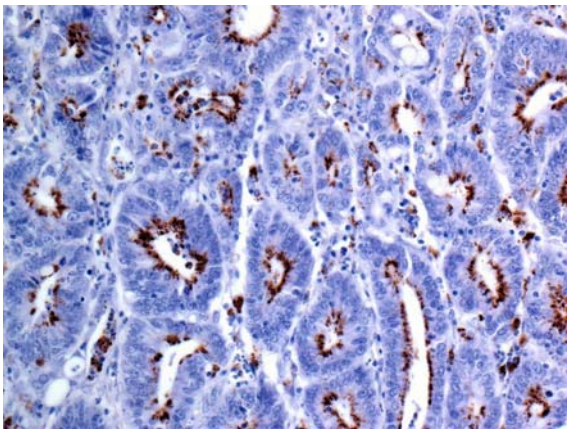

Figure 4. IHC. Intracellular *Lawsonia intracellularis* antigen (red-brown) in enterocytes of a pig with ileitis.

AVIAN/FUR/EXOTIC SPECIES

Lameness in 7 day old broilers

Emily Martin, David Ross

In April of 2013, the AHL was contacted to discuss a case of broiler chickens with a history of lameness at 7 days of age. Significant numbers of birds showed mild to moderate valgus deviation of both legs with increased size variation of affected birds. The birds were reluctant to move and mortality was 2.1-2.2 %. The affected birds were located on the second floor of a 2-storey barn. The birds on the first floor were not displaying clinical signs. The 2 floors were managed by different people, were contracted by different companies, and had different feed suppliers.

Clinical examination revealed that the birds did not have obvious swelling of the joints, and some birds had lesions suspicious for bacterial septicemia. The possibility of bacterial infection, reovirus infection and testing options were discussed. Three broiler birds were submitted for postmortem examination as well as fixed tissues for histopathology and swabs for bacterial culture.

On gross postmortem examination of the 3 submitted birds, 1 bird had bilateral swelling of the hock joints and footpads, and 1 bird had lateral extension of both legs. All 3 birds had feed material in the crop and gizzards. After thorough examination, it was found that the keel bones, tibiotarsal bones, and beaks were easy to bend. This raised the suspicion of rickets. In order to rule out an underlying infectious etiology, multiple tests were ordered including bacteriology (on submitted bacteriology swabs), reovirus PCR on closed hock joints (AHL postmortem samples), and histopathology.

On histopathology, there were two groups of tissues to examine: those submitted by the referring veterinarian and those collected on postmortem at AHL. There were few findings in the multiple tissues examined from each bird. In the submitted tissues, there was a single section of spinal column with meningitis and there was cellulitis in the skin overlying the hock/tendon sections. In the tissues collected on postmortem at the AHL, there was very mild synovitis in 1 section of bone composed of scattered lymphocytes and plasma cells. The bones were difficult to examine due to the normal mass of cartilage that persists in the metaphysis of the long bones in poultry up to 7 - 14 days of age (Figure 1). However, the general impression of bone histology was that **there were increased numbers of osteoblasts and decreased numbers of osteoclasts vaguely suggestive of phosphorus deficiency rickets** (Figure 2). Feed analysis was recommended and already underway.

On bacterial culture of the 2 submitted swabs, occasional *E. coli* were isolated from the hock swab and large numbers of *E. coli* were isolated from a peritonitis swab. The reovirus PCR was positive, however, considering that there were no consistent lesions on histopathology to support this diagnosis, it was suspected that this was not the primary cause of the lameness.

Analysis of feed samples taken from bin augers indicated excess calcium and low phosphorus levels, confirming the diagnosis of rickets. Results showed calcium levels of 134-140%, and phosphorus levels of 72-75% of expected values. This particular type of rickets is not commonly identified in clinical samples at the AHL. Deficiency of calcium and vitamin D is more commonly diagnosed in the cases of rickets submitted to the AHL. *AHL*

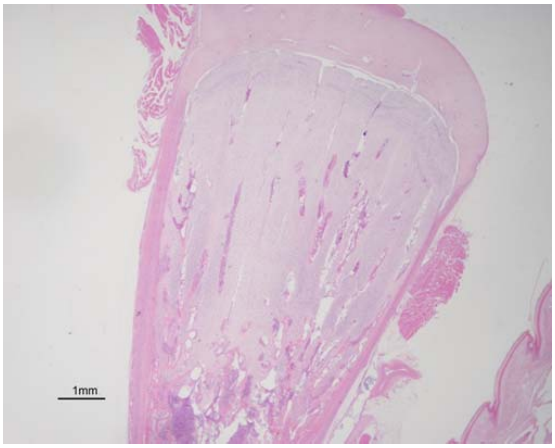

Figure 1. Longitudinal section of a proximal tibiotarsal bone showing the normal cone of cartilage that persists in the metaphysis of long bones in poultry up to 7 to 14 days of age. 1X

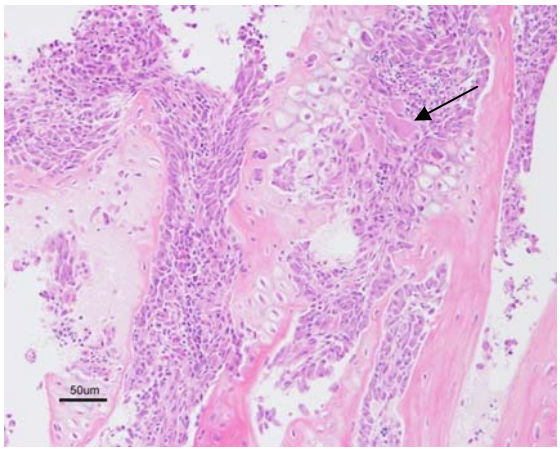

Figure 2. Higher magnification of the cartilage cone in the metaphysis showing vascular channels lined by numerous osteoblast and rare osteoclasts (arrow). 20X

HORSES

Equine arteritis virus testing *Davor Ojkic*

Infection with equine arteritis virus (EAV) is often subclinical and infected horses recover uneventfully. In acutely infected horses, the virus is spread horizontally and can cause interstitial pneumonia in young foals and respiratory infection of adult horses. Unprotected pregnant mares are at a risk of abortion and/or can give birth to congenitally infected foals with rapidly progressive pneumonia. Infection of stallions post-puberty can result in a long-term carrier state. During breeding, persistently infected stallions carry the virus in their reproductive tract and transmit EAV to susceptible mares to precipitate new outbreaks. There is only one serotype of EAV; however, EAV strains differ in their virulence.

The AHL offers 2 tests for diagnosis of EAV infections:

- **Polymerase chain reaction test for detection of EAV** in blood, serum, plasma, tissues, nasal swabs ($42.00/sample).
- **Virus neutralization test for detection of antibodies** against the virus in serum ($15.00/sample).

Tests are offered weekly, or more often depending on demand. For time-sensitive submissions, please contact the lab in advance at ahl.virology@uoguelph.ca *AHL*

Ontario Racing Commission Death Registry: 2003-2012 postmortem summaries *Josepha DeLay*

The Ontario Racing Commission Death Registry has been in place for 10 years and continues to provide excellent data regarding the causes of morbidity and mortality in racehorses in this province. Since 2008, there has been a shift in emphasis to non-fracture cases, most with increased complexity. Summaries of postmortem submissions to the AHL under this program and diagnoses by body system for these cases are provided in the following tables. *AHL*

Table 1. Breed distribution of ORC Death Registry postmortem submissions to the AHL, 2003-2012.

Year / Breed	Standardbred	Thoroughbred	Quarter Horse	Total
2003	67 (54%)	58 (46%)	0	**125**
2004	82 (58%)	60 (42%)	0	**142**
2005	59 (54%)	51 (46%)	0	**110**
2006	58 (54%)	47 (44%)	2 (2%)	**107**
2007	66 (54%)	53(43%)	3(3%)	**122**
2008	27 (53%)	24(47%)	0	**51**
2009	28 (62%)	16 (36%)	1 (2%)	**45**
2010	22 (69%)	8 (25%)	2 (6%)	**32**
2011	24 (52%)	18 (39%)	4 (9%)	**46**
2012	20 (59%)	14 (41%)	0	**34**

Table 2. Postmortem diagnoses of ORC Death Registry submissions by body system, 2003-2012. Note change in emphasis of case selection for postmortem submissions beginning in 2008, as mentioned in text above.

Diagnosis by body system:	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Fracture / limbs	53 (42%)	69 (49%)	48 (44%)	42 (39%)	54 (44%)	16 (31%)	4 (9%)	9 (28%)	6 (13%)	2 (6%)
Fracture / other	10	4	7	13	10	5	0	3	6	2
Non-fracture musculoskeletal	8	6	6	8	6	5	2	3	1	0
Gastrointestinal	15	19	17	16	18	5	4	7	5	6
Respiratory (including EIPH)	21	17	9	11	16	9	21	6	9	7
Cardiovascular	5	6	5	5	2	4	6	2	4	1
CNS	6	11	7	4	1	1	2	0	5	4
Integumentary	0	0	1	2	2	1	1	0	0	0
Renal	0	2	0	0	2	0	1	0	0	0
Hematopoietic	2	1	1	0	0	0	0	0	0	0
Whole body conditions (e.g., septicemia)	1	7	5	2	9	0	4	0	6	6
Cause of death undetermined	4 (3.2%)	0 (0%)	4 (3.6%)	4 (3.7%)	2 (1.6%)	5 (9.8%)	0 (0%)	2 (6%)	4 (9%)	6 (18%)
Total	**125**	**142**	**110**	**107**	**122**	**51**	**45**	**32**	**46**	**34**

COMPANION ANIMALS

Mycobacterium avium complex (MAC) infection in a cat

Kristiina Ruotsalo

A 12-year-old, male, neutered, domestic shorthaired cat was presented to the referring veterinarian with a **history of acute dyspnea**. Thoracic imaging revealed pleural effusion and a mass associated with the right pleural surface. Malignancy was clinically suspected.

Direct smears from the mass as well as concentrated fluid preparations were examined. The slides from the mass exhibited hemorrhagic, proteinaceous backgrounds. Large numbers of inflammatory cells were present, consisting of approximately 80% macrophages and 20% lytic neutrophils. The macrophages contained abundant, negatively staining, fine, rod-shaped organisms consistent with *Mycobacterium* sp. (Figure 1). Extracellular organisms were also found free within the slide backgrounds. Slides from the pleural fluid preparations revealed a predominantly suppurative inflammatory response with rare macrophages containing intracellular, negatively staining organisms.

Acid fast staining of additional slides confirmed large numbers of positive intracellular and extracellular organisms consistent with *Mycobacterium* sp. (Figure 2). Due to the potential human health risk, remaining fluid was submitted to the Public Health Laboratory for further testing. Their final result revealed the presence of *Mycobacterium avium* complex.

One group of mycobacteria includes saprophytes that behave as **opportunistic pathogens**, causing localized or systemic disease, depending upon the degree of host compromise. This group can be further divided into **slow growers** including ***Mycobacterium avium* complex (MAC)**, and **rapid growers**. MAC organisms are ubiquitous worldwide in soil and water under conditions of acidic pH (5.0 to 5.5), and high organic matter. Feces of infected birds contain large numbers of bacilli and infection of dogs and cats is thought to occur from ingestion of infected meat or contact with infected soil or fomites contaminated by poultry carcasses or feces. MAC organisms can remain viable for at least 2 years in the environment, including municipal water supplies, soil, dairy products, and tissue of birds and mammals. Despite the widespread nature of MAC organisms, infection in humans, dogs, and cats is rare. In cats, localized lymphadenitis as well as disseminated disease has been reported. In dogs, granulomas often form in visceral organs with only rare dissemination.

Previous case reports of disseminated MAC infection in cats have included over-representation of young Abyssinian and Siamese cats, leading to speculation that a breed - or line-specific immunodeficiency might be predisposing to infection. Other cases have included cats with either a positive FeLV or FIV status, receiving immunosuppressive agents (cyclosporine following renal transplantation), or presumed infirmity associated with malignancy, malnutrition, endocrinopathy or splenectomy.

This case was unusual because this cat had no clearly identifiable source of potential infection. He lived in a single cat household and had been housed strictly indoors with no known exposure to birds, household plants or soil. He had not received any potentially immunosuppressive medications and had no concurrent documented illnesses. His FeLV/FIV status was negative. **Despite attempted antimicrobial therapy, the cat's condition quickly deteriorated and he was euthanized.** *AHL*

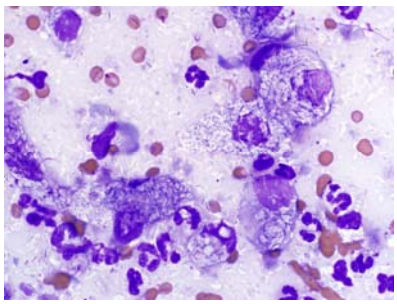

Figure 1. Wright's-stained smear from pleural mass showing macrophages filled with negatively staining mycobacteria.

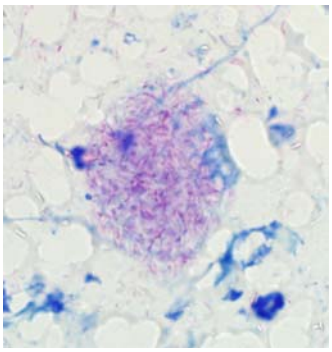

Figure 2. Acid fast stained mycobacteria in a macrophage.