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

AHL Newsletter

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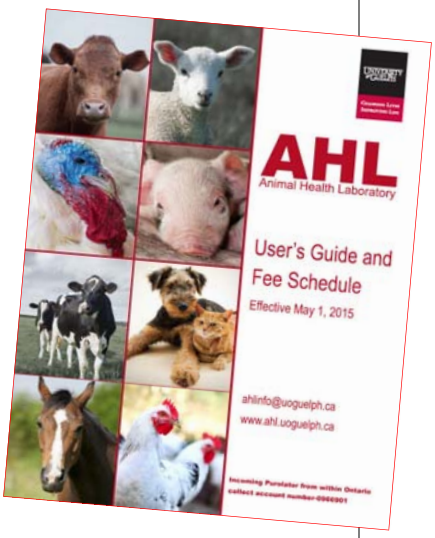

Effective May 1, 2015 we have updated our tests and fees. We've added in various new or modified tests over the past year: Porcine coronavirus (PEDV, TGEV, PDCoV), triplex PCR; Orf virus/Papular stomatitis virus, PCR; virus sequencing (Canine distemper virus, Canine parvovirus 2/ Feline panleukopenia virus); Feline calicivirus/Felid herpesvirus 1, PCR; fish viral hemorrhagic septicemia virus (VHSV), PCR; fish bacterial identification by MALDI-TOF MS; *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, duplex PCR.

We have inactivated many older or less popular tests, such as virus isolation and fluorescent antibody testing, in favor of more suitable PCR tests.

Our complete test list is available in our May 1, 2015 AHL User's Guide and Fee Schedule recently sent out to our clients. For a searchable test listing and pricing, our clients can visit us at our website at www.ahl.uoguelph.ca If you do not have client access for fees – please contact ahlinfo@uoguelph.ca , or call 519 824 4120 ext. 54320 and we will be more than happy to help you with this or anything else you might require.

Once again, we look forward to serving you! *AHL*

AHL staffing update

Michael Deane, joins us as our new Communications Assistant in the Ontario Animal Health Network, supporting Dr. Melanie Barham, our OAHN Coordinator, under the Disease Surveillance Program (DSP).

Liz King has assumed the role of Quality Manager for Lab Services Division, following the recent retirement of Nadine Ryan. Liz, with a staff of 4, oversees the ongoing maintenance of the LSD quality program, and the whole range of LSD quality accreditations—ISO/IEC 17025, AAVLD, and OECD GLP.

Pauline Nelson-Smikle is the new Information Technology Manager for Lab Services Division, replacing the retiring Phyllis Few. Pauline, with a staff of 4, ensures the ongoing functionality of all LSD IT infrastructure, including our LIMS (Lab Information Management System, i.e., LabVantage or Sapphire).

"Influenza D" in cattle and swine? Keeping an eye out for new diseases…

Davor Ojkic, Anna Marom, Murray Hazlett

A novel influenza virus with 50% homology to human influenza C viruses was isolated from a pig during an outbreak of influenza-like illness in Oklahoma, and 9.5% of laboratory submitted porcine serum samples were serologically positive when tested subsequently (1). This virus was later demonstrated via PCR from nasal swabs in 18% of cattle with respiratory disease (45 tested), and was found serologically to be common in most bovine herds (2).

The proposal is to call this orthomyxovirus "influenza D virus". We did a brief survey of recent respiratory disease submissions looking for involvement of this novel virus. Histology scrolls for PCR testing were prepared from histology blocks – including bovine pneumonic lung (15 blocks), porcine pneumonic lung (15 blocks), and bovine and porcine abortion tissues (5 blocks each – used as controls). In addition, 15 miscellaneous non-fixed bovine samples were tested (7 lung, 3 mucosal swabs, 2 serum samples, and 1 fetal lung/tissue pool.

No "influenza D" virus was identified in any of the samples tested. This was admittedly a very small study, and is part of our ongoing disease surveillance efforts. The test may be periodically requested by pathologists in unusual or suspect cases however it will not be available as a routine test.

References

1) Hause BM, et al. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoS Pathog 2013;9:e1003176.
2) Hause BM, et al.. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the *Orthomyxoviridae* family. MBio 2014;5:e00031-14.

OAHN podcasts

- ◊ Melanie Barham and Mike Deane are adding continuously to our stock of podcasts.
- ◊ For practical information on the go! 10 programs in stock.
- ◊ Posted at ahn.podbean.com
- ◊ Our latest, "**Photographing lab samples in the field - practical tips for veterinarians**":
- ◊ http://oahn.podbean.com/e/photographing-lab-samples-in-the-field-practical-tips-for-veterinarians/

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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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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.

Making sense of *Salmonella* spp. serotyping results

Durda Slavic *(From AHL LabNote 35, May, 2015)*

At the AHL, all clinical *Salmonella* isolates are sent for serotyping to the Laboratory for Foodborne Zoonoses (LFZ) because of **public health concerns**. As a rule of thumb, if *Salmonella* spp. are isolated from multiple samples from the same farm, their colony morphology is examined. If they look similar, only one colony is selected for serotyping. If morphology differs, multiple colonies are sent. *Salmonella* isolates are sent to LFZ every Wednesday and it usually takes **2-4 weeks** to receive serotyping and phagetyping results back. It should be noted that phagetyping is done only for *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Heidelberg.

Salmonella taxonomy underwent a major overhaul in 2005 when ***Salmonella enterica*** and ***Salmonella bongori*** (formerly group V) were established as the only 2 species of salmonellas. At the same time, it was recognized that the *S. enterica* group is comprised of 6 subspecies:

Salmonella enterica subsp. *enterica* (group I)
Salmonella enterica subsp. *salamae* (group II)
Salmonella enterica subsp. *arizonae* (group IIIa)
Salmonella enterica subsp. *diarizonae* (group IIIb)
Salmonella enterica subsp. *houtenae* (group IV)
Salmonella enterica subsp. *indica* (group VI)

Differentiation of *Salmonella* **species** and **subspecies** is relatively straightforward and it can be done **biochemically**. However, within each *Salmonella* subspecies, isolates can be further divided into different serotypes. **Serotyping** of salmonellas is based on **immunological detection** of 2 groups of cell surface antigens: lipopolysaccharides (**O antigens**) and flagellin proteins (**H antigens**). Moreover, H antigens in salmonellas are usually expressed as phase 1 and phase 2 antigens, a feature unique to *Salmonella* sp. As a result, each *Salmonella enterica* serotype has a specific antigenic formula shown as a combination of letters and numbers. This **formula** consists of **subspecies designation** (i.e., I, II, IIIa, IIIb, IV, VI), **O antigens** (i.e., a number or combination of numbers), **phase 1** (i.e., lower case letter), and **phase 2 flagellin** (i.e., lower case letter or combination of numbers) **antigens** (Fig. 1). When all of the antigens are detected for a specific serotype of group I salmonellas, then that serotype is reported by a name. For example, *Salmonella enterica* subsp. *enterica* will be reported as serotype Typhimurium only if the following antigens are detected: S. I 4,5,12:i:1,2 (Fig.1). If any of these antigens are not detected, then that particular isolate will be reported by its antigenic formula only (e.g., S. I 4,5,12:i:-). In contrast to group I salmonellas, serotypes belonging to groups II through VI are always reported by their antigenic formulas only (e.g., S. IIIa 51:z_4,z_{23}:-).

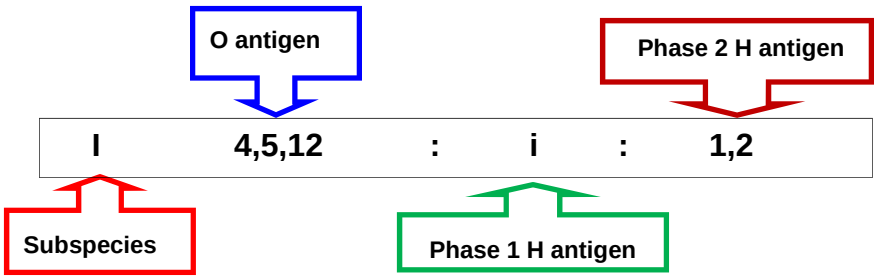

Figure 1. Antigen designation of *Salmonella* Typhimurium in the *Salmonella* sp. serotyping scheme.

To translate this into a practical perspective when you are looking into *Salmonella* serotyping results - if there is a name reported, it means that this particular serotype belongs to group I and that all antigens defining that serotype were detected. If there is an antigenic formula only, the first thing that one needs to look at is the subspecies designation. If there is a subspecies designation from II through VI, the full serotype is reported and there is not much additional information to be inferred from the results. However, if an antigenic formula for subspecies I is reported, in some cases more information can be gathered including **serotype variants**.

A variant that we see most frequently in the lab is a **monophasic *S.* Typhimurium** with antigenic formula **S. I 4,5,12:i:-.** This antigenic formula defines a variant of *S.* Typhimurium missing a phase 2 H antigen. Some other variants of *S.* Typhimurium are shown in Table 1. *AHL*

Table 1. Variants of *Salmonella* Typhimurium and their antigenic formulas.

Designation	Antigenic formula
Monophasic	S. I 4,5,12:i:-
Nonmotile	S. I 4,5,12:nonmotile
Rough	S. I rough:i:1,2

Ontario Animal Health Network

Your comprehensive source for animal health information."

OAHN Update

The Ontario Animal Health Network is progressing well. We have made significant progress with a number of expert networks, which you can view below:

	CORE COMPONENTS							
	Assemble experts	Lay groundwork	Assemble network	First cycle	Industry engagement	Second cycle	Network maturation	Annual meeting
Small ruminants								
Swine								
Poultry								
Fish								
Wildlife								
Fur-bearing/alt								
Equine								
Bovine								
Bees								
Companion								

In addition to the Network progress, we have also set up a temporary webpage: http://oahn.ca, while our official page is created. We expect the design of the page to be complete by mid-June. We are also excited to include provincial slaughter condemnation data in the major agricultural sectors, as an additional information source. We are recording some great podcasts currently, so stay tuned at www.oahn.podbean.com, or subscribe via iTunes to never miss an episode. You may also wish to follow our Facebook and Twitter feeds for the most up to date information about disease outbreaks and network releases. If you have a suggestion/idea, let us know! Network updates are below.

SHEEP/GOATS

Q1 of 2015 (Jan/Feb/March) has completed
Look out for the Q1 2015 vet and producer reports on SRVO listserv.
Check out our podcast with OSMA and Ontario Goat (see link below).
Top items of discussion: emaciation and associated disease due to harsh winter.

FISH

Dr. Melanie Barham spoke about OAHN at the recent Northern Ontario Aquaculture Association meeting in Parry Sound.
Q2 2015 fish meeting will be taking place mid-June.

BOVINE

The bovine network is up and running!
Q1 2015 veterinary report will be released soon to the OABP listserv. We had 71 responses to the survey after emailing the OABP listserv. AHL contributed data, and provincial slaughter data was also included.
Dr. Melanie Barham spoke about OAHN at the recent OABP/OABA joint meeting.

BEES

The first OAHN bee meeting will be taking place in late June 2015. Date and time TBA.
We will be releasing a bee podcast shortly with information of interest to the general public, veterinarians and bee keepers.

WILDLIFE

CCWHC is preparing a report for distribution to all the groups outlining the details of their surveillance systems in Ontario. Stay tuned for the reports in coming months.

SWINE

Q 1 of 2015 (Jan/Feb/March) has completed.
Look out for the Q1 2015 vet and producer reports on the OASV listserv.
Gallant Custom Laboratories contributed data alongside AHL data.
Top items of discussion: colibacillosis in suckling piglets, ascarid issues causing liver condemnation at provincial slaughter plants.

EQUINE

Q1 2015 veterinary report was released to OAEP listserv.
IDEXX laboratories will be contributing data alongside AHL in the next quarter.
Ontario Racing Commission death registry data will be included in the next quarter.
The first survey had 69 responses after emailing the OABP listerv.
Discussions about anthelmintic resistance led to an AHL newsletter article about testing, and an owner report from OMAFRA (to be released soon).
OAHN will be participating in the first equine disease simulation being hosted with OMAFRA mid-June.

COMPANION

The companion animal network will be launched imminently. A call for nominations will be put out within the next month.
A great group of veterinarians across Ontario met as a focus group to help determine the shape of OAHN's companion animal network.
Follow us on Facebook for up to date notifications about diseases in your area, and helpful news articles for you and your clinic.

POULTRY

Q2 2015 (Feb/March/April) meeting is taking place May 21st- expect the veterinary report shortly thereafter.
Avian influenza infographic for backyard flock owners can be found on our Facebook page, and was well received by industry.
OAHN was featured on multiple radio broadcasts for farm radio reports, directing producers to resources.
Veterinarian to veterinarian mentoring was set up through CVO and participation of Ontario Poultry vets.
OAHN held an emergency call early in the avian influenza outbreak to discuss possible gaps and issues of concern. Small flock grower initiatives were undertaken together with OMAFRA and industry, including release of avian influenza information to small flock growers, information for vets called upon to treat small flocks, and teleconferences for small flock growers with experienced poultry veterinarians.

ALTERNATIVE SPECIES

Dr. Hugh Hildebrandt, leading expert in mink medicine, lead a well-attended mink veterinary workshop April 11th, 2015. The workshop was attended by 15 veterinarians from across the province and some via teleconference from across Canada. Drs. Marina Brash, Emily Martin, Pat Turner were also instructors for the post mortem workshop, and Brian Tapscott from OMAFRA lectured about the background and future of this growing sector. Industry partners generously shared the cost of this workshop.
A call for mink veterinarians to discuss cases with experts in the field will be taking place each quarter. If you are interested in being part of this call, please contact Dr. Melanie Barham at barhamm@uoguelph.ca

AHL Lab Reports

RUMINANTS

A blackleg outbreak in a group of dairy heifers

Murray Hazlett, Matthew Walker, Durda Slavic

In April, over a period of 2.5 weeks, **6 Holstein heifers from a closed herd died.** They had been clinically normal the night before, and were found dead, or moribund less than 12 hours before death. All had received 2 doses of routine respiratory vaccines, but no clostridial vaccines. They were kept in a large free-stall area on sand with about 45 heifers in the group. There was no unusual handling, although some animals were coming into heat periodically and may have been mounting others. There had been some recent excavation in order to expand the milking parlor. Farm management was excellent.

Two animals were submitted to the AHL for autopsy, one that had died (8 months old), and another that was euthanized (12 months). Gross examination was similar in both animals. There was some blood draining from the nares, and one heifer was somewhat bloated. An ELISA for anthrax was performed on both and was negative. On opening the bodies, in the first animal, there was hemorrhage with emphysema involving a 20 x 20 x 6 cm area in front of the right scapula (Fig. 1). A 2 x 2 x 4 cm region of dark hemorrhage was present in the left ventricular free wall papillary muscle. There was marked acute hemorrhage and emphysema with areas of "dry" pallor of the adductor muscles of the right and left hind leg and in tissue dorsal to the udder. Approximately 2,400 cm^3 of muscle were involved. In the second animal, there was hemorrhage with some emphysema involving a 10 x 20 x 6 cm area in the brisket region and acute hemorrhage and emphysema of the adductor muscles of the right hind leg – ~1,200 cm^3 being affected. Microscopically there was myofiber degeneration with coagulative necrosis. There was marked interstitial edema and emphysema, and in areas a sparse infiltrate of degenerate neutrophils was seen (Fig. 2). Large numbers of gram-positive clostridial-type organisms were present in the section. In the necrotic areas, many myofibers were devoid of nuclei and there were scattered foci of hemorrhage.

Smears done on fresh muscle stained with fluorescein-conjugated antibodies demonstrated *Clostridium chauvoei* in large numbers in both animals, with no staining for *C. novyi*, *C. septicum*, or *C. sordelii*. No bacterial pathogens were isolated on culture.

Blackleg "outbreaks" have been associated with excavation (1) and spores have been recoverable in contaminated soil at 11 years (2) and perhaps longer. It is also associated with trauma, and assumed to be activation of dormant spores in damaged muscles. **In this case, trauma may have been associated with estrus activity, or the "outbreak" may have been due to the recent excavations.** The remaining heifers have since been vaccinated for clostridial diseases, and there have been no subsequent losses.

References

1) Barnes DM, et al. Selected blackleg outbreaks and their relation to soil excavation. Can Vet J 1975;16:257-259.

2) Raducanescu H, Bica-Popii V. Persistence of *C. chauvoei* spores in various types of sterile soils. Archiva Vet 1967;3:227-234.

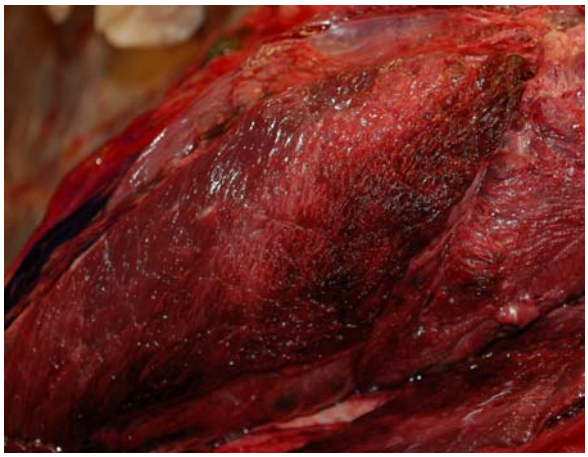

Fig 1. Autopsy photo of *Clostridium chauvoei* infected muscle showing emphysema, hemorrhage, edema, and necrosis.

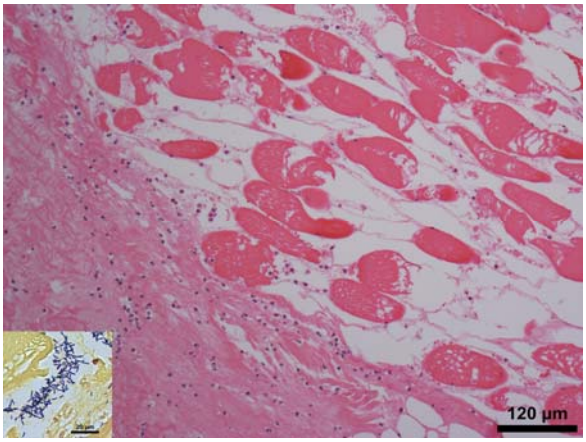

Fig 2. Histology of involved muscle – degenerate myocytes and an infiltrate of degenerate leukocytes in some areas of the lesion are typical. Close of up Gram stain shown in the insert.

Streptococcus suis isolates from arthritis lesions in Ontario kids and lambs

Margaret Stalker, Durda Slavic, Beverly McEwen, Allyson MacDonald, Emily Mergo

Case 1: In January 2015, a 3-day-old lamb was submitted for postmortem from a flock of 100 in confinement housing. Sixty animals were affected, 2 dead, with a history of swollen joints, non-responsive to a range of treatments including tetracycline in milk, A180® danofloxacin, prednisone, Banamine®, Baytril®, or Liquamycin LA-200®. Lambs were kept with the ewe for 2-3 days, then separated and started on milk replacer. Within 1-2 days, the lambs developed swollen joints. No difference in rate of infection was noted, whether the navels were dipped or not. A 1-week-old lamb posted on-farm a week prior to the current submission had 3+ *Streptococcus suis*, and *E. coli* (the latter on enrichment culture only) isolated from a joint swab. The lamb submitted for postmortem at the AHL had a markedly swollen left carpus containing dry, inspissated yellow exudate, and enlarged and edematous left prescapular and axillary lymph nodes. Histology revealed marked fibrinosuppurative synovitis with extension of the inflammation into the adjacent carpal bone. Cultures for *Mycoplasma* were negative. Bacterial cultures revealed **4+ *S. suis* from the carpus, and 3+ from the prescapular lymph node**. The isolate was sent for serotyping, and typed as *S. suis* serotype 9.

Case 2: In February 2015, a 7-day-old goat kid was submitted for postmortem from a herd of 800 milking does. Many of the young kids (20/40) were developing lameness with swollen joints, and were unresponsive to antibiotic therapy with tetracycline or lincospectin/isoflupredone acetate. In the affected group, 15% had more than one joint affected. Two kids had concurrent conjunctivitis and 2 kids had pneumonia with pyrexia. Kids at this farm were snatched at birth, given pasteurized cow colostrum, and then fed powdered milk replacer. All navels in affected and non-affected kids were clean and dry. On postmortem examination, the kid was found to have a swollen left carpus, with inspissated fibrin and yellow caseous exudate in the proximal carpal joint. **1+ *S. suis* was isolated from the carpal swab**; cultures for *Mycoplasm*a were negative. The isolate was sent for serotyping, but was untypeable (i.e., not serotypes 1-34). Two weeks after these test results became available, a new group of does on this farm started kidding. Six kids from this cohort of birthing does started to show signs of conjunctivitis and lameness. Before joint swelling was noted, they were treated with florfenicol/flunixin and none progressed to have swollen joints; all recovered normally.

***S. suis* isolates from cases 1 and 2 were resistant to tetracycline and tilmicosin, with intermediate susceptibility to erythromycin.**

Streptococcus suis is an important pathogen of swine, causing sudden death, meningitis, septicemia, arthritis, endocarditis, and pneumonia. It has also been increasingly isolated from a range of other animal species including ruminants, horses, dogs, and cats. A recent case report from the Atlantic Veterinary College documents *S. suis* arthritis in 2 lambs from a single premises (1). *S. suis* (particularly serotype 2 but also other serotypes) (2), is also a potential zoonotic pathogen.

The AHL database from May 2007-February 2015 was queried (Table 1). Arthritis, pulmonary abscessation, and abscesses in various locations were the most frequent diagnoses in juvenile sheep and goats < 6 months of age. Joints involved included carpal, tarsal, and stifle joints. One lamb had a concurrent umbilical infection with *S. suis*. *AHL*

References

1) Goyette-Desjardins G, et al. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent – an update on the worldwide distribution based on serotyping and sequence typing. Emerg Microbes Infect 2014;3:e45 online.

2) Muckle A, et al. Isolation of *Streptococcus suis* from 2 lambs with a history of lameness. Can Vet J 2014;55:946-949.

Table 1: *Streptococcus suis* infections in sheep and goats, May 2007-May 2015

Diagnoses	**Sheep (n=13)**	**Goats (n=14)**
Arthritis , < 3 months old	6 (1* with *Ureaplasma*; 1* with *E. coli*)	3
Pneumonia/pulmonary abscesses, < 3 months old	2 (1 with *Mannheimia* and mycoplasma)	3 * (1 with myocarditis)
Meningitis, 2 weeks old		1
Conjunctivitis, 4 days old		1*
Vertebral abscess, 6 months old	1	
Pharyngeal abscess, 2 months old	1*	
Thyroid abscess, 1 month old	1*	
Abscess, cheek/jaw/site unknown, adult or age unknown	1*	3*
Mastitis/milk samples, adult animals	1	3* (2 bulk tank samples)

* = in mixed culture

Erosive and fibrinous polyarthritis caused by *Ureaplasma diversum* in bovine fetuses

Maria Spinato, Andrew Brooks, Hugh Cai

Two bovine fetuses submitted to the AHL for postmortem examination had unusual joint lesions attributed to in utero *Ureaplasma diversum* infection.

Case 1 involved a Holstein heifer fetus aborted at approximately 8 months gestation. The abortion was an isolated case within the herd and the cow was healthy. At postmortem examination, the fetus had severe arthritis affecting multiple joints including the hips, shoulders, carpi, and hock joints. The affected joints contained thick exudates, erosion of the articular cartilage, and hyperemia of the synovial lining. The lungs of the fetus were aerated, indicating that it was alive at the time of abortion. Histologically, the joints exhibited marked arthritis with fibrin exudates, hyperplasia of the synovium, and infiltrates of neutrophils, lymphocytes, and plasma cells (Fig. 1). The fetus also had mild encephalitis and hepatitis. *Ureaplasma diversum* was isolated from an affected joint and lung.

Case 2 involved a Holstein bull calf that was born 28 days prematurely. The animal had difficulty standing after birth, and was noted to have a swollen left foreleg. At one week of age, the calf developed diarrhea and neurologic signs and subsequently died. At postmortem examination, the animal was noted to be small, weighing only 26 kg. Thickened joint capsules and fibrotic periarticular tissue were noted in the left carpal, right stifle (Fig.2), and atlantoaxial joints. Affected cartilage appeared yellow, pitted and fibrillated. Abundant fibrinopurulent exudate filled the foramen magnum and surrounded C1 spinal cord. The caudal aspect of vertebra C1 and the cranial aspect of C2 had extensive irregular erosion of articular cartilage, and the dens appeared collapsed and deformed (Fig.3). Histologically, sections of thickened synovium had prominent villus hyperplasia and lymphoplasmacytic infiltrates with focal effusion of fibrin into the joint space. Affected appendicular bone sections revealed irregular thinning of articular cartilage with loss of matrix and chondrocytes. Vertebra C1 contained several concentrically oriented clusters of degenerate neutrophils and macrophages surrounded by irregular deposits of mature fibrovascular stroma. Cervical spinal cord segments had mild Wallerian degeneration of ventral white matter tracts, consistent with compressive myelopathy. A section of eyelid revealed mild conjunctival epithelial hyperplasia and mucous metaplasia with dispersed subconjunctival lymphoplasmacytic infiltrates. Cultures of lung and right hock synovium were negative for pathogenic bacteria and *Ureaplasma* spp. in this calf.

A recent case series of 5 aborted bovine fetuses described destructive polyarthropathy attributed to in utero *Ureaplasma diversum* infection which was identified by PCR or culture in 4 of the 5 fetuses (1). Gross and histologic lesions in this paper are similar to those described in these 2 affected calves. Cows infected with *Ureaplasma diversum* typically abort in the 3rd trimester; premature or weak term calves may also occur. This organism is part of the normal genital tract flora of cattle, and is believed to be transmitted during breeding. *Ureaplasma* has also been isolated from artificial insemination and embryo transfer fluids. Common histologic lesions are amnionitis and chorioallantoic vasculitis in the placenta, and lymphoplasmacytic conjunctivitis with goblet cell metaplasia and non-suppurative interstitial pneumonia in fetal tissues (2). **Joints are rarely examined closely when performing postmortems on bovine fetuses, and it is possible that this lesion has been overlooked in some cases of abortion caused by *Ureaplasma diversum*.** *AHL*

References

1) Himsworth CG, et al. Destructive polyarthropathy in aborted bovine fetuses: A possible association with *Ureaplasma diversum* infection? Vet Pathol 2009;46:269-272.

2) Schlafer DH, Miller RB. Female genital system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol.3, pp. 429-564. Elsevier, Edinburgh, UK, 2007.

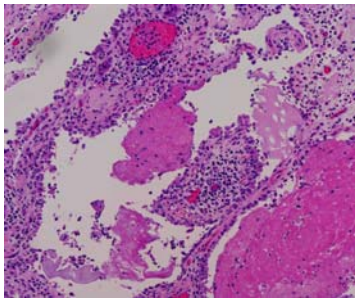

Figure 1. Histologic section of joint containing hyperplastic synovium, fibrin exudate, and inflammatory cell infiltrates (case 1).

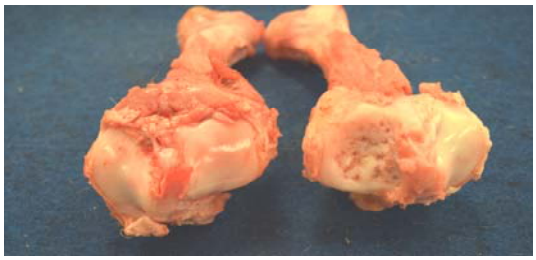

Figure 2. Thickened and pitted articular cartilage over right lateral tibial plateau compared to left (case 2)

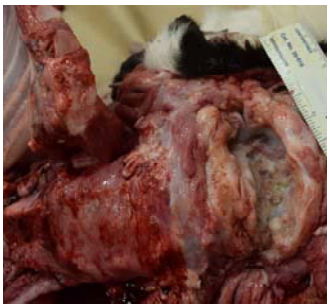

Figure 3. Thickened joint capsule, deformed dens, and fibrinosuppurative exudate in atlantoaxial joint (case 2).

HORSES

How to choose an equine parasitology test

Melanie Barham, Mary Lake

When choosing an equine fecal parasite detection method, it is important to determine the goal of the test. Are you:

- ◊ Deworming after the winter?
- ◊ Performing fecal Egg Count Reduction testing to look for anthelmintic resistance?
- ◊ Trying to find tapeworm eggs?
- ◊ Deciding to treat an individual animal or a herd?

There are 3 major tests used in the equine industry:

McMaster fecal egg count: This test uses a quantification technique that allows the veterinarian to know the number of eggs per gram (epg) of feces, and involves using a specific counting/quantification slide. It is the recommended method for screening populations of horses to determine which require treatment. The technique is sensitive to 50 epg (each egg detected on the slide is calculated to be equal to 50 epg) and all types of parasite eggs identified are reported. The sensitivity of this test is quite appropriate for most fecal testing prior to treatment or in situations where a high load of parasites are suspected, and is significantly less expensive than the Modified Wisconsin technique.

Modified Wisconsin technique: The Modified Wisconsin technique is another fecal quantification technique that is sensitive to 1 epg and is more sensitive for tapeworm eggs than a McMaster, although will still only pick up 15-20% of tapeworm eggs. As with the McMaster technique, all types of parasite eggs identified are reported. The cost per test is higher than the McMaster test as 2 centrifugations are required. In cases where there is a high fecal egg load, or a high load is suspected, this technique is not ideal, as eggs can become too numerous to count, and may require McMaster testing first with a secondary scan for tapeworm species via the Wisconsin technique, increasing the cost. It is recommended to use this type of testing in fecal egg count reduction tests (FECRT), or in situations where previous treatment has not alleviated signs.

Standard gravitational fecal flotation: The fecal flotation test is the traditional test for fecal parasite eggs, but does not quantify exact numbers of eggs per gram. Again, all parasite eggs identified are reported, but because exact quantification is not performed, results are reported as 1+, 2+, or 3+.

A note about tapeworms: if a tapeworm parasite load is suspected, sampling 10 or more horses on the farm is advised, and submitting for Wisconsin testing will yield the best results. Experimental testing by fecal ELISA is being studied, but is not currently available for commercial use.

Sample submission tips:

- ◊ All fecal tests give the best results when samples are collected and submitted within 24 hours of the horse passing the feces.
- ◊ Immediate and continual refrigeration in an airtight container prior to submission significantly improved results in research trials, and samples collected need only be 1 fecal ball in size; 5 g is all that is required for samples.
- ◊ Samples can be refrigerated for up to 7 days before analysis, although immediate submission is best.
- ◊ Fecal samples should be submitted in fecal containers.
- ◊ We are happy to send containers to you at no charge: containers typically arrive within 24-48 hours of your request.

A comprehensive document outlining treatment and test interpretation has been released by the American Association of Equine Practitioners and can be referenced here: http://www.aaep.org/custdocs/ParasiteControlGuidelinesFinal.pdf

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SWINE

Porcine coronavirus update *Jim Fairles*

There has been considerable activity in PEDV testing over the winter months, and we hope that spring and summer will once again aid in the reduction of virus in the province.

AHL recognizes all of the organizations that are contributing to the reduction and elimination of PEDV in Ontario particularly Ontario Swine Health Advisory Board, Ontario Pork, and the Ontario Ministry of Agriculture, Food and Rural Affairs.

Current statistics (available courtesy of OMAFRA at www.ontario.ca/swine) indicate to date, **17 premises with an initial clinical diagnosis since the fall of 2014.** The great reduction in cases from the initial outbreaks last winter, we hope is related to the fine work the industry is doing in biosecurity.

The AHL encourages veterinarians to submit samples from any cases of undifferentiated diarrhea. (see Labnote 30 - http://www.guelphlabservices.com/AHL/LabNotes.aspx)

To aid in biosecurity, we recommend that the triplex porcine coronavirus PCR for PEDV, TGEV, PDCoV (deltacoronavirus) be done *BEFORE* submitting animals to the lab for postmortem where diarrhea is a clinical sign and coronavirus is suspected - the triplex PCR test will continue to be done at no cost to clients as part of OMAFRA/AHL disease surveillance for the initial clinical diagnosis (Ontario submissions only).

Some statistics on initial clinical diagnosis cases are listed below. These have been funded by the Disease Surveillance Program – money allocated by OMAFRA from federal Growing Forward 2 money to the AHL for work in disease surveillance. http://www.uoguelph.ca/omafra_partnership/en/partnershipprograms/DiseaseSurveillancePlanDSP.asp and www.oahn.ca

Initial clinical diagnosis statistics

Total number of case accessions since the outbreak began – 1037 cases

Total number of samples tested – 6,121

	Q1 2014	Q2 2014	Q3 2014	Q4 2014	Q1 2015	Q2 2015 to date
samples	4,488	504	204	222	421	196

AVIAN/FUR/EXOTIC SPECIES

Avian influenza prevention for mixed practitioners *Melanie Barham*

Many practitioners come across small flocks on farm visits, or office visits in the cases of pet chickens or birds etc. If you or your clients have questions about avian influenza and its prevention, there are many resources available. www.oahn.ca has resources for veterinarians who may be called upon to treat small flocks, including history questions, prevention tips and fact sheets. **A number of experienced poultry veterinarians across Ontario volunteered to help mentor colleagues not used to dealing with poultry species; this mentor list can be accessed by calling the CVO registrar's office.** Chicken Farmers of Ontario also has some excellent resources for small flock growers, and posts up to date information for all small flock owners (http://www.ontariochicken.ca/Programs/Small-Flock.aspx). OMAFRA additionally has excellent biosecurity tips that are practical for non-commercial growers and pet bird owners alike. A free small-flock kit can be ordered by any bird owner through OMAFRA or Poultry Industry Council. (http://www.omafra.gov.on.ca/english/livestock/poultry/health.html)

A few quick tips for all veterinarians:

- **If a high level of bird mortality is noted on any farm, please call your local CFIA office immediately.**
- Small flock growers should limit access of wild birds to their commercial flocks as the wild birds can harbor disease without clinical signs.
- The avian influenza virus is spread via aerosol as well as fomites such as boots, trucks, clothes and hair, and has a relatively long survival time. It is effectively killed with products such as Virkon.
- If visiting a farm where birds of any kind (wild or domestic) are present, and traveling to another farm where birds are located, you should change clothes, including footwear, if you have come in contact with bird droppings or areas where birds are located.
- Although the current outbreak numbers appear to be stable in Ontario, as migratory birds travel south in the fall, the disease may resurface. *AHL*

Mink workshop *Melanie Barham*

On April 11th, 2015, the Animal Health Laboratory, together with OAHN and several industry sponsors, hosted a workshop for veterinarians interested in learning about mink medicine. 17 veterinarians attended from across Ontario, and several from out of province via teleconference. **Dr. Hugh Hildebrandt,** expert mink veterinarian from Wisconsin, was the main speaker. Other speakers included **Mr. Brian Tapscott** of OMAFRA, who lectured about the mink industry and its future, and basics of nutrition and feeding. **Mr. Dre Sanders** of National Feeds gave a detailed lecture about mink nutrition.

The afternoon portion of the laboratory involved hands on mink post mortem, lead by **Drs. Marina Brash, Emily Martin** (AHL) and **Pat Turner** (OVC). Other sponsors for the event included Canadian Mink Breeders Association, Ontario Fur Breeders Association, National Feeds, and MVRCS.

COMPANION ANIMALS

Canine influenza *Andrew Vince*

Canine influenza is caused by an **H3N8 influenza A virus**, and has been recognized intermittently since an outbreak of respiratory disease in racing Greyhounds in 2004. In that first outbreak, initial cases occurred in Florida and eventually spread to involve 30 states and Washington DC.

In 2015, a new outbreak of respiratory disease in Chicago led to the identification of an **H3N2 strain of influenza A virus**, apparently related to strains of canine influenza circulating in Korea, China, and Thailand since 2006, notably responsible for an outbreak of respiratory disease in dogs in Thailand in 2012. Current strains in Illinois and Iowa are reportedly closely related genetically to Asian strains, although a clear point of introduction to the United States has not been identified. There is no indication of an outbreak of canine influenza in Canada as of this report.

Clinical signs of canine influenza infection include fever, sneezing, nasal discharge, dry cough, and loss of appetite lasting 5-7 days.

The virus is extremely contagious; the American Veterinary Medical Association has estimated that as many as 80% of dogs exposed to the virus will be successfully infected. However, **case fatality rates have been very low**, with rare mortalities limited to those dogs with other co-morbidities. These viruses are typically shed for 7-10 days, beginning 24 hours prior to the onset of clinical signs.

Several recommendations have been posed by the CVMA and AVMA, including:

- If you think your dog has influenza, **contact your veterinarian before bringing your dog to the clinic**. Most infected dogs will not require veterinary care. If your dog does become severely ill, it may need medical treatment, but contacting your veterinarian allows them to take steps to mitigate risk for other animals
- If possible, keep your dog away from other dogs if it is ill or has been exposed to ill/infected dogs, for 7-14 days.
- If you live in an area where the virus becomes endemic, diminish dog-dog contact while the virus is spreading (such as dog parks), and avoid travelling with your dog outside that area.
- Avoid purchasing/importing dogs/puppies from endemic areas.

Influenza A matrix PCR testing is optimal for the diagnosis of canine influenza in both antemortem and post-mortem samples, requiring only submission of a nasopharyngeal swab in virus transport medium; tissues (principally lung) are also a useful postmortem sample. It is strongly suspected that the AHL immunohistochemical stain could aid in the postmortem diagnosis of canine influenza (based on prior detection of related viruses in other species), although the lack of Canadian cases has left this untested. Hemagglutination inhibition (HI) testing is of limited utility in diagnosis of acute disease, as it is most limited by strain variations and depends on the presence of antibodies, which may not be present until later in the progression of disease. *AHL*

More resources:

CVMA website: "Canine Influenza: information for veterinarians and dog owners" (http://www.canadianveterinarians.net/news-events/news/canine-influenza-info-for-vets-and-dog-owners), accessed 20 May 2015

Worms and Germs Blog: "More on Canine H3N2 Flu" (http://www.wormsandgermsblog.com/2015/04/articles/animals/dogs/more-on-canine-h3n2-flu/), accessed 20 May 2015

OVMA website: "Canine Influenza Information" (http://www.ovma.org/extra/canine_flu_info.html), accessed 20 May 2015

Clostridial myositis resembling pseudoblackleg in a cat

Andrew Vince

The body of a 6-year-old cat was received for postmortem investigation at the Animal Health Laboratory. The cat had been seen by a veterinarian with an initial 1-hour history of repeated vomition and lethargy, had a fever of 40.5°C, but otherwise no significant abnormalities on physical examination. Bloodwork was recommended and declined, and the cat was treated with Convenia and Cerenia and sent home for monitoring and recheck the day following. The cat was examined again the following day , was still febrile, and rapidly became recumbent and stuporous. The cat began to act painfully and started dragging its right hindlimb, and there was palpable swelling and gas crepitation extending from the toes to the pelvis. The cat progressed rapidly to coma and cardiopulmonary arrest.

At postmortem, the right hindlimb was diffusely edematous, congested, and palpably emphysematous. The skeletal muscle of the right hind limb was irregularly dark red-black, dry, and friable (Fig. 1), with a characteristic odor (typically described as "rancid butter"). The lungs were diffusely firm, rubbery, and wet. Histologically, there was generalized hemorrhage and edema throughout the muscle and soft tissues of the right hindlimb; skeletal myocytes were swollen and hypereosinophilic, with rare sarcoplasmic fragmentation and vacuolation. Small numbers of neutrophils were noted in the interstitium. There was also generalized pulmonary edema, focal adrenal necrosis, and limited emphysema within the adrenal and spleen. Muscle from the right thigh was submitted for bacterial culture and clostridial fluorescent antibody testing (FAT), both of which **demonstrated large numbers of *Clostridium septicum* in pure culture**.

Clostridium septicum is a gram-positive, spore-forming, obligate anaerobic bacterium that can cause cellulitis and myositis without the need for percutaneous inoculation. In most species, it is postulated that infection is established through hematogenous spread from the gastrointestinal tract; myonecrosis then occurs as a result of the release of several exotoxins, including alpha, beta, gamma, and delta toxins.

Clostridium septicum is best described as a cause of acute fatal myositis and septicemia (called "pseudoblackleg") in cattle, small ruminants, horses, and swine, with only a few cases described in companion animal and zoo/wildlife species. **Pseudoblackleg is characterized by the absence of a detectable inoculation wound**. In this case, and in most similar companion animal reports, pure culture of this bacterium and lack of obvious wounds resulting in infection strongly suggests a hematogenous origin, although the possibility of latent infection and the factors that might activate such an infection are poorly understood. In similar cases, death is typically rapid after onset of clinical signs, even with prompt treatment. Culture and/or FAT on tissue with consistent lesions of myonecrosis and emphysematous myositis is an effective way of confirming this diagnosis. **Clostridial myositis should be suspected with the triumvirate of necrosis, hemorrhage, and tissue emphysema in soft tissues and muscle, regardless of animal species affected.** *AHL*

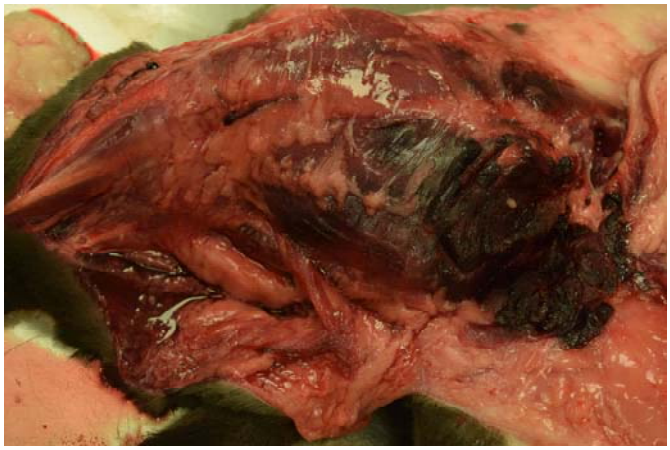

Figure 1. Proximal right thigh of a cat with clostridial myositis. Muscle is regionally purple-black, and edema fluid is pooling in fascia.

Encephalitozoon cuniculi microsporidiosis in a 10 week old puppy

Margaret Stalker, Jennifer Lillywhite, Hugh Cai, Pat Bell-Rogers, Rebeccah McDowall, Qiumei You

A 10-week-old female Boxer puppy was received for postmortem examination, following a brief period of illness characterized by vomiting, laboured breathing, pale mucous membranes, hypothermia, and collapse, with rapid clinical deterioration culminating in death. The pup had been present in its new home for 3 days prior to the onset of clinical signs, and had received DAPPV vaccination 5 days prior, as well as periodic deworming with Strongid. The remaining 8 littermates were reported to be healthy.

On postmortem examination, the heart had faintly visible irregular areas of pallor visible on the epicardial surface, with moderate dilation of the right atrium and ventricle. Histologic examination revealed a widespread, multisystemic inflammatory disease characterized by infiltrates of macrophages, lymphocytes, plasma cells, and in some areas neutrophils, particularly evident in the myocardium, kidneys, and brain. Kidney sections had occasional medullary tubular epithelial cells with clear cytoplasmic vacuoles containing multiple, faintly staining ovoid organisms, ~1-3 µm long (Fig. 1). These organisms did not stain with antibodies to *Neospora caninum* or *Toxoplasma gondii*, and were PAS-negative, however, they did stain with Brown & Brenn tissue Gram stain (Fig. 2). Two gel-based PCR assays for *Encephalitozoon cuniculi* (1, 2) were strongly positive. The PCR product of each assay was sequenced and found to have 100% and 99% identity with the *E. cuniculi* small subunit ribosomal RNA gene. Further strain characterization using primers designed to amplify the internal transcribed spacer (ITS) region of the rRNA allowed further identification of the organism as ***E. cuniculi* genotype II**.

E. cuniculi is an obligate intracellular spore-forming parasitic fungal organism belonging to the phylum Microsporidia, and is best known as a relatively common parasite of rabbits and rodents, where it typically causes subclinical infections. A recent case series documented 19 cases of fatal encephalitozoonosis in puppies from Texas (3), and confirmed the identity of the organism as *E. cuniculi* strain III. Affected puppies ranged in age from 4-10 weeks, and had brief clinical histories of depression, inappetence, and neurologic signs. On postmortem, renal lesions were consistently found, characterized by lymphoplasmacytic and histiocytic interstitial nephritis with organisms visible in renal tubular epithelial cells, as in this case. Brain lesions were also typically found, with *E. cuniculi* spores in vascular endothelium in the brain parenchyma.

E. cuniculi* has recently been divided into 3 strains**: strain I infects rabbits and humans; strain II infects mice, rats, and blue foxes; and strain III infects dogs, swine, and humans, although the full range of susceptible species continues to expand. **Infections in humans are typically in immunocompromised individuals, highlighting the potential zoonotic risk of these organisms**. The life cycle is direct, and the primary route of transmission is through ingestion of infective spores shed in urine by infected hosts. Horizontal transmission is implicated in the Texas study, where most infected animals were associated with breeding facilities containing numerous dogs. Transplacental transmission from asymptomatic dams has also been documented experimentally in dogs. Clinically significant infections are generally seen in puppies, likely acquired from the dam. No effective treatment has been reported for dogs. In this case, the source of infection was undetermined in this puppy, but thought likely to be from an asymptomatic infection in the dam. **This case represents the first report, to our knowledge, of *E. cuniculi* infection in a dog in Canada, and the first report of *E. cuniculi* strain II infection in a dog.** ***AHL

References

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2) Meng X, et al. First characterization in China of *Encephalitozoon cuniculi* in the blue fox (*Alopex lagopus*). J Eukaryot Microbiol 2014;61:580-585.
3) Snowden KF, et al. *Encephalitozoon cuniculi* infections in dogs: A case series. J Am Anim Hosp Assoc 2009;45:225-231.

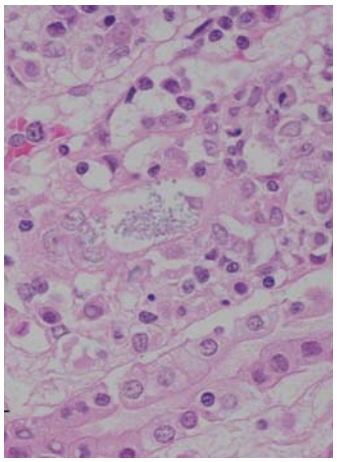

Figure 1: Organisms in kidney, H&E stain.

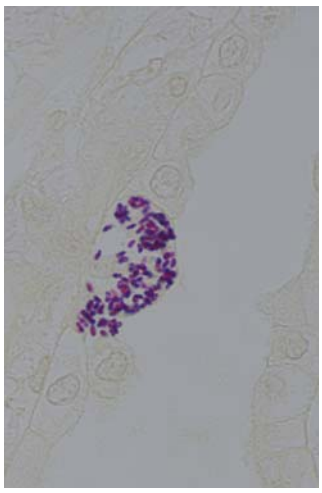

Figure 2: Organisms in kidney, B&B Gram stain.