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

AHL Newsletter

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In this issue:

Season's Greetings from the staff of the Animal Health Laboratory

AHL Holiday Hours, 2015/16

Closed Christmas Day, Dec 25.
Otherwise, open with limited services.
Guelph and Kemptville drop box and/or refrigerator are available 365/24/7 for specimen drop off.
Guelph - **Usual Saturday services** include: specimen receiving, emergency mammalian autopsies, full bacteriology set up, as well as clinical pathology testing. **Statutory holiday services and usual Sunday services** include: specimen receiving, emergency mammalian autopsies, and basic bacteriology set up.
For full details, please see our website—ahl.uoguelph.ca

Update on the Ontario Non-commercial Poultry Flock Disease Surveillance Project *Marina Brash, Leonardo Susta, Michele Guerin, Csaba*

Despite the ever-increasing numbers of non-commercial poultry flocks in Ontario, we know very little about the disease prevalence, biosecurity, and husbandry practices of these flocks. In order to understand the management practices and to assess the baseline prevalence of relevant infectious diseases (viruses, bacteria, parasites) in non-commercial flocks in Ontario, *OMAFRA* and the *University of Guelph*, through the *Animal Health Laboratory (AHL)* and the *Ontario Animal Health Network*, have started a **2-year surveillance study that will run until September 29, 2017.**

For the substantially discounted fee of $25.00 per submission - plus a completed submission form, husbandry questionnaire, and a signed participation consent form - owners of small flocks of chickens, turkeys, gamefowl, geese, and ducks, are encouraged to **submit sick or dead birds, through their veterinarian, to the AHL in Guelph or Kemptville for postmortem examination and infectious agent testing**. Results from this project will be used in developing educational tools to improve the health and welfare of backyard flocks.

For further details, visit http://www.guelphlabservices.com/AHL/Poultry_Flock_Disease.aspx or http://phrn.net/dis-surveillance-dr-susta-lab/, or contact Dr. Marina Brash at 519-824-4120 x54550, email: mbrash@uoguelph.ca or Dr. Leonardo Susta at 519-824-4120 x54323, email: lsusta@uoguelph.ca

Cold weather shipping reminder *Jim Fairles*

It's that time of year again when we need to start thinking about preventing samples from freezing. Specimens such as EDTA blood are rendered useless when frozen. Formalin will also freeze, which creates artifacts in fixed tissue. It can be difficult to protect samples shipped during the winter from severe cold. Even 10% neutral-buffered formalin will freeze in harsh winter weather conditions. **To prevent formalin freezing, add 1 mL of ethanol per 10 mL of formalin.**

Samples that should not be frozen should be shipped inside insulated containers with minimal cold packs. *Use of room temperature cold packs will help prevent temperatures from dipping too low.* If you have any concerns about the best way to ship critical samples, please contact the AHL. ahlinfo@uoguelph.ca

New services from AHL Molecular Biology/Mycoplasmology *Hugh Cai*

With the support of the OMAFRA Disease Surveillance Plan (DSP), the AHL Molecular Biology/Mycoplasmology Section has implemented the following assays:

- **18S rRNA gene sequencing** for fungal identification from culture, fresh, fixed or frozen samples
- ***Batrachochytrium* PCR** for the identification of *Batrachochytrium dendrobatidis* and *B. salamandrivorans,* which case chytridiomycosis, a lethal fungal disease of amphibians.
- ***Echinococcus multilocularis* and *E. granulosus* PCR** for tissue and ascites samples.
- **Fish bacterial culture and identification** using MALDI-TOF MS, PCR and DNA sequencing.
- **Fish postmortem** and wet mount examination; fish histopathology (Dr. Nikki LePage).
- **Fish viral PCR** for viral hemorrhagic septicemia virus (VHSV). The assay is accredited by ISO17025 under the AHL flexible scope.
- **Honey bee viral and vitellogenin PCR** will soon be ISO17025 accredited under the AHL flexible scope. Our honey bee testing services cover the detection of the following pathogens and biomarkers: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), sacbrood virus (SBV), *Nosema apis*, *N. ceranae, Spiroplasma apis, S. melliferum, Crithida mellificae, Lotmaria passim, Apocephalus borealis*, *Tropilaelaps* species (screening), *Varroa destructor* mites (haplotyping), vitellogenin mRNA (a marker of overall honey bee health).
- ***Mycoplasma iowae* qPCR** is now accredited with ISO17025 under the AHL flexible scope.
- ***M. hyopneumoniae mhp183* gene qPCR** which is an upgrade from a gel based PCR with better sensitivity and shorter turnaround time.
- ***Mycoplasma meleagridis* qPCR** for avian samples.
- ***Mycoplasma species* PCR**, which uses an ATCC kit to identify over 60 *Mycoplasma species* mainly for detection of cell cultures mycoplasma contamination.
- ***Ophidiomyces ophiodiicola* qPCR** for snake fungal disease diagnosis.
- ***Small hive beetle (SHB)* qPCR and DNA sequencing** for confirmation of SHB ID of insects and larvae.
- ***Streptococcus equi subsp. equi eqbE* gene qPCR**, which is more sensitive than the original *seM* gene PCR.

For further information please contact ahlinfo@uoguelph.ca

AHL Newsletter
December, 2015 - Volume 19, Number 4

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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.

Ontario Animal Health Network

"Your comprehensive source for animal health information."

OAHN Update December 2015

OAHN is happy to announce that all 10 animal networks are now active! Welcome to the family companion animal network!! Other items of note this quarter:

- We had a very successful 3rd Annual Meeting of the Disease Surveillance Plan on Oct. 1, which saw private vets and industry members work with OMAFRA, OVC, AHL, and other government members to form an action plan for the network's coming year.
- Each OAHN network is invited to submit a project proposal to address a gap in surveillance (budget $50,000 per network). Deadline is December 1st. If you have ideas, contact your OAHN network members from your species group today.
- We continue to put out 2 podcasts per month. Check out titles here: www.oahn.podbean.com
- We continue to share disease alerts, scholarly articles, client friendly animal health news on our website, and through our Facebook and Twitter accounts (@OntAnHealthNet).

SMALL RUMINANTS

The Q3 2015 veterinary survey was completed and the quarterly conference call occurred at the end of October. Look out for the veterinary and producer reports on the SRVO listserv and on OAHN.ca . **Top items of discussion were: Bluetongue surveillance by OAHN, upcoming captive bolt lab with industry, a recent case of ovine adenocarcinoma in Quebec, and fewer cases of hemonchosis this year.**

FISH

The OAHN Fish Network had its 4th quarterly conference call in October. The group is working towards submitting their project proposal, and discussed pertinent fish disease issues in ONT.

BOVINE

The OAHN bovine network had its Q2 quarterly call in September. The report outlined recent findings of *Salmonella* Dublin in Quebec and resources for practitioners, as well as an increased number of IBRV cases. The next call will be in early January 2016. Look for the survey invite in the coming weeks.

BEES

The bee network will be meeting before the end of December 2015 to summarize the year's disease events. Stay tuned for further information.

SWINE

The OAHN swine network completed its Q3 2015 veterinary survey and had its quarterly conference call in October. Look out for the swine veterinary report and producer report to be published on OASV listserv and on OAHN.ca in the coming

EQUINE

The Q3 equine veterinary and owner reports were released to the OAEP listserv and posted on OAHN.ca in November. **The top concerns discussed during the Q3 conference call were: increased diarrhea cases in Eastern Ontario, and fevers of unknown origin. Some veterinarians reported fewer equine strangles cases compared to the same quarter last year, and reported neuro disease cases were lower this year.** Antimicrobial resistance information from AHL is referenced in the report, and is further detailed in the AHL newsletter. AHL, IDEXX, and the Ontario Racing Commission contributed data.

ALTERNATIVE SPECIES

The OAHN alternative species network held its first conference call in July, with mink veterinarians discussing important issues with experts in the field. **Main points of discussion for the call involved astrovirus and nursing sickness**. To be added to the list for future calls, email oahn@uoguelph.ca.

POULTRY

The OAHN Poultry Network had its Q4 conference call in November. The Q3 2015 (May/June/July) meeting took place in September, and the producer report is available on OAHN.ca. **Top issues for discussion during the Q3 conference call included: white chick syndrome, coccidiosis, necrotic enteritis, bacterial and fungal infections.** OMAFRA, OVC, the AHL, and OAHN started a small flock disease surveillance project on Oct. 1, which includes subsidized testing for non-commercial poultry owners. An OAHN representative presented at OAPV meetings in September and November.

WILDLIFE

The Canadian Wildlife Health Cooperative released a report on wildlife disease surveillance in the late fall of

COMPANION ANIMALS

The OAHN companion animal network had its first quarterly conference call to discuss quarterly survey results in October. Disease issues discussed included a public health/OMAFRA update on *Echinococcus multilocularis* cases, as well as the recent respiratory disease outbreaks in dogs in Orangeville and other parts of the province. Stay tuned for the veterinary report in the next month.

Want to receive veterinary reports? Email oahn@uoguelph.ca

AHL Lab Reports

RUMINANTS

Subclinical copper toxicity in Ontario dairy cows

Brent Hoff, Nick Schrier, Beverly McEwen

Chronic copper toxicity has classically been associated with chronic overprovision of copper (Cu) to sheep. There and only a few reports of similar cases in cattle, suggesting that cattle have some degree of resistance to Cu toxicity.

An increasing frequency of Cu toxicity has been reported in European dairy cattle. Pathologists from Minnesota and Wisconsin report several cases of subclinical Cu toxicity in high-producing Holstein dairy cows, with **clinical signs of infertility and ketosis refractory to treatment with propylene glycol.** Cases had diffuse hepatocellular vacuolation, random, single-cell necrosis, biliary hyperplasia, and mononuclear inflammation with levels of Cu >150 ppm wet weight (ww) (normal reference interval 25 -100 ppm) in livers. When the liver is damaged, energy mobilization and the cow's ability to adapt to negative energy balance changes, with the main impact on fertility and possibly milk yield. Liver damage, as determined by elevated serum levels of the leakage enzyme glutamate dehydrogenase (GLDH), began at 150 ppm ww of Cu in the liver, and decreased following withdrawal of dietary Cu. It has also been reported that dietary supplements leading to Cu accumulations in the liver at concentrations only slightly above normal levels (~125 ppm ww) caused reduced feed intake and average daily gain.

Subclinical Cu toxicity is often difficult to detect, and may be a more frequent problem in high-producing dairy cattle than the minimal number of clinical cases would indicate. Low serum Cu is of some value in chronic deficiency of non- supplemented beef cows, as the hepatic Cu has been depleted, but is of no value in supplemented daily cows. Unfortunately, **only assays on liver biopsies from high-producing dairy cows will confirm elevated Cu levels because the association between serum Cu and liver Cu is poor**.

Review of AHL ICP-MS trace mineral analyses on liver of 102 adult cows revealed a mean Cu of 118 ± 66 ppm ww (normal reference interval 25-100 ppm) with 25 samples >150 ppm with a mean Cu of 205±74 ppm.

Liver biopsy samples are an especially good means of assessing Cu status. They are also very useful in assessing selenium status and histopathology. We can measure trace mineral concentrations in very small liver biopsy samples that can easily be taken using a "Tru-Cut" type biopsy instrument. These small samples can be placed in the bottom of a small nylon container and quickly frozen. The liver biopsy procedure can be found in LabNote 19 on the AHL website: http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote19.pdf *AHL*

Changes in the cutoffs for milk and serum Johne's antibody tests

Jim Fairles, Davor Ojkic

Cow milk samples tested for paratuberculosis (Johne's disease) by Canwest DHI and serum samples tested by the Animal Health Laboratory at the University of Guelph use the same IDEXX ELISA kit. Recently, **IDEXX revised the cut-offs for negative, suspect and positive test results**. These changes are intended to increase the sensitivity of the test slightly, and **will take effect December 1, 2015**.

The table below includes the new cut-offs for both milk and serum, as well as the old cut-offs for comparison. The cut-off for a high-positive test result in both milk and serum remains unchanged at sample-to-positive (S/P) ≥ 1.0. *AHL*

Milk - New			**Milk - Old**		
Negative	Suspect	Positive	Negative	Suspect	Positive
S/P ≤ 0.20	0.20 < S/P < 0.30	S/P ≥ 0.30	S/P ≤ 0.30	0.30 < S/P < 0.40	S/P ≥ 0.40
Serum – New			**Serum - Old**		
Negative	Suspect	Positive	Negative	Suspect	Positive
S/P ≤ 0.45	0.45 < S/P < 0.55	S/P ≥ 0.55	S/P ≤ 0.60	0.60 < S/P < 0.70	S/P ≥ 0.70

SWINE

Senecavirus A in pigs *Josepha DeLay, Jim Fairles, Davor Ojkic*

Senecavirus A (Seneca Valley virus, SVV) has recently been associated with clinical disease in pig herds in Brazil (2014 and 2015) and in the midwestern United States (summer 2015). Testing has identified a low prevalence of infection in the US. The virus was identified at an assembly site in Canada in October 2015, but concurrent clinical disease has not yet been documented in this country. Prior to 2015, sporadic cases of SVV infection were identified in both the US and Canada.

Sows and finisher pigs infected with SVV develop cutaneous vesicular lesions on the snout and coronary bands. The lesions may progress to ulceration and sloughing of the hoof wall, and affected animals may be lame. Morbidity is high, however there is no direct mortality associated with SVV infection in older pigs, and affected pigs generally recover in 1-2 weeks. Vesicular lesions in SVV-infected pigs are clinically identical to those of several federally reportable foreign animal diseases (FADs), including foot and mouth disease (FMD), swine vesicular disease (SVD), and vesicular stomatitis of swine (VSS). In contrast to FADs, infection with SVV is not a federally reportable disease in Canada. **However because of the similarity of lesions, FAD exclusion is a priority in any herd where vesicular lesions are identified, and the Canadian Food Inspection Agency (CFIA) must be notified immediately when these lesions are detected**. It is imperative that pigs with any evidence of vesicular or ulcerative lesions involving snout or hooves NOT be shipped for slaughter. Presence of vesicular lesions at slaughter inspection would result in closure of the plant while FAD investigation is carried out. This would have a significant negative economic impact on the Ontario and national swine industry, as well as on individual producers.

In addition to vesicular lesions described in older pigs, recent cases in the US and Brazil have documented disease in neonatal pigs associated with high tissue levels of SVV. Clinically, there was a dramatic spike in mortality (up to 70%) among 1-4 day old neonates, with significant but lower mortality among 5-7 day old piglets. The increase was transient, resolving in 7-10 days. Vesicular lesions were present concurrently in 70% of sows; in the remaining 30% of cases, clinical disease was limited to piglets. The syndrome of neonatal pig infection has not been previously described in association with SVV, and has been dubbed '**epidemic transient neonatal mortality**' (ETNM). Diarrhea is present in some affected piglets, and may be the result of concurrent infection with various other pathogens including rotavirus enteritis and *Clostridium difficile* colitis. No specific gross or histologic lesions in affected piglets have been attributed to SVV infection.

High levels of SVV have been identified in vesicle fluid from pigs with cutaneous lesions. In addition, SVV antigen and nucleic acid were identified in multiple tissues from piglets in cases of ETNM, leading to the suggestion that death is the result of acute viremia. Despite this association, Koch's postulates have not yet been fulfilled to confirm a causal role for SVV in either vesicular disease or neonatal mortality. Features of SVV incubation, shedding, and transmission are also uncharacterized at this time. Based on information from the Brazil outbreak, SVV does appear to be highly immunogenic, with development of long-lasting immunity.

Testing for SVV is dependent on the clinical syndrome identified in the herd. For cases with vesicles, CFIA must be contacted and will direct testing in order to exclude FADs. Testing for SVV will be included in the investigation. In herds experiencing a sudden increase in neonatal mortality but WITHOUT vesicular lesions in sows or other animals, please contact the Animal Health Laboratory (AHL) for direction regarding sampling and test selection. Samples required from neonates include serum, and both formalin-fixed and fresh samples of multiple tissues (lymph nodes, tonsil, spleen, liver, lung, kidney, heart, small and large intestine, brain, and spinal cord, if possible).

The AHL, in collaboration with the CFIA National Centre for Foreign Animal Disease (NCFAD), is validating a PCR test for SVV. This test will be available in soonfor use in any situation with no clinical evidence of vesicular disease, such as environmental monitoring and neonatal mortalities. Serological testing methods are

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Influenza A virus testing from swine *Janet Alsop*

From each submission to AHL that tests positive for Influenza A virus by PCR, one sample will be selected for further typing and results of this additional testing will be reported to clients:

a. H1N1/H3N2 typing; b. Partial H gene sequencing

On unusual viruses (i.e., non H1N1/H3N2), viruses associated with outbreaks and for emergency purposes (e.g., H5/H7), whole genome sequencing will be periodically carried out. For this testing, samples will be batched to minimize the cost and the results will be reported on a cumulative basis, i.e. no individual identification of source herd.

OMAFRA will cover all test costs apart from the initial PCR.

AVIAN/FUR/EXOTIC SPECIES

What are 'white striping' and 'wooden breast' in broiler chickens?

Emily Martin, Alexandra Reid, Marina Brash

Breast muscle myopathies are emerging worldwide in the broiler industry, but more recently in North America. Two forms of breast muscle myopathy are known as 'white striping' and 'wooden breast'. Both affect the pectoralis major (breast) muscle and are described in several fast-growing commercial broiler strains. Carcasses with breast muscle myopathies result in economic losses because of carcass downgrading (sent for further processing), condemnation of whole birds or portions, or impact consumer choice.

White striping is grossly described as visible white lines of variable quantity and thickness running parallel to the muscle fibers (Fig. 1) - **these lines are composed of adipose tissue**. In severe cases, white striping can also have increased connective tissue and variable degrees of myofiber degeneration and regeneration.

Wooden breast is described grossly as a hardening of the breast muscle, primarily in the proximal fillet (pectoralis major) but can affect the whole muscle. Other observations include pallor, surface hemorrhages, and a sterile surface exudate. Histologically, **there is polyphasic myodegeneration with regeneration and cellular infiltration with increased interstitial adipose and connective tissue** (Fig. 2)

White striping and wooden breast can be found either alone or in combination. Opinions vary as to whether these are 2 distinct myopathies or whether they represent a disease spectrum with white striping as the initial lesion progressing to wooden breast. **There is no clear etiology for these myopathies**. Etiologies that have been examined include, vitamin E or selenium deficiency, associated pathogens, genetics, and environmental factors.

In wooden breast, the findings are not consistent with nutritional myopathy given that the pectoralis muscle is hard on palpation and there are no lesions noted in other muscle groups such as the gizzard or heart.

The leukocyte counts in white-striped birds do not differ from unaffected birds suggesting that there is no systemic infection. In cases of wooden breast, even though there appears to be an exudate over the breast, no associated pathogens have been reported.

In earlier studies, researchers developed a theory that growth selection resulted in larger diameter muscle fibers with decreased capillary blood supply to the muscle, reduced connective tissue spacing between muscle fibers and bundles, and increased myofiber degeneration. Physiologically, the breast muscles are composed of fast-twitch aerobic muscles that, as part of anaerobic respiration, produce lactic acid that is removed in the circulation. With reduced connective tissue spacing, there are fewer capillaries and less lactic acid being removed from the tissues. Lactic acid build-up could result in decreased pH, muscle damage, and satellite cell mediated regeneration. However, a more recent genetic study revealed that the selection for breast yield may have some role. Environmental factors (i.e. nutrition, management) may play a significant role in the expression of the myopathies.

Muscle fiber formation is complete at hatch, therefore muscle growth is related to the satellite cells associated with the existing muscle fibers. In the hatchery, increased incubation temperatures at certain times may influence myoblast activity and breast muscle yield. Further study is required to determine if this impacts the later development of myopathies. Once the birds are in the barn, there are multiple studies that indicate that feed restriction (during the first 2 weeks of life or at 13-21 days of age) resulted in a change in satellite cell activity associated with increased muscle fiber necrosis and fat deposition in the pectoralis major muscle. Another study showed that feeding of low-energy diets reduced both growth rate and the occurrence of white striping.

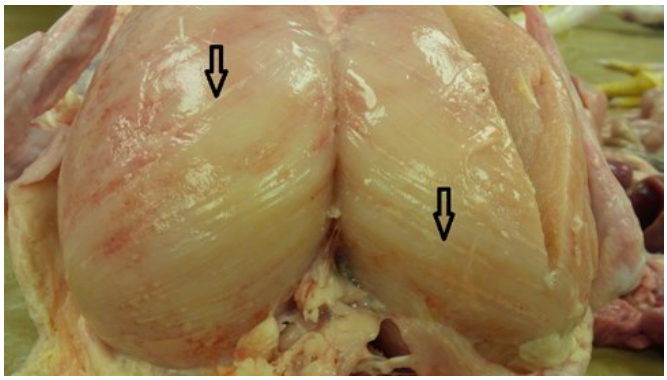

Figure 1. **White striping** (arrows) in breast muscle (photo courtesy of Dr. Alexandra Reid, OMAFRA).

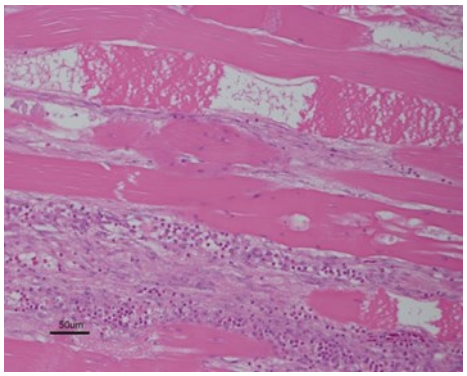

Figure 2. Histologic section of breast muscle showing polyphasic myodegeneration and interstitial fibrosis consistent with **wooden breast** (20X).

Overall, the lesions of white striping and wooden breast result in product quality issues that are

HORSES

Antimicrobial susceptibility pattern of selected equine pathogens from clinical cases submitted to the AHL between May 2007 and May 2015

Durda Slavic, Murray Hazlett, Beverly McEwen, Michael Deane, Melanie Barham

The use of laboratory testing (culture and antimicrobial susceptibility panels), plus prudent counselling of owners, can assist in stewardship of antimicrobial use. To assist decision-making, we searched the AHL laboratory information system for the **susceptibility patterns of common equine pathogens for the past 8 years** (Fig. 1). Results were available for 155 *Actinobacillus equuli* (*A. equuli* subsp. *equuli* and *A. equuli* subsp. *haemolyticus*), 382 *Escherichia coli*, 792 *Streptococcus equi* subsp. *zooepidemicus,* and 131 *Streptococcus equi* subsp. *equi* isolates.

There was a **high level of resistance in beta-haemolytic *Streptococcus* spp. to aminoglycosides**, including amikacin and kanamycin, and a lower level of resistance to gentamicin. As expected, ***Streptococcus* spp. isolates were sensitive to penicillin**, which should be a primary drug of choice for their treatment. **The susceptibility patterns for *E. coli* and *A. equuli* were less predictable**, with resistance being present to all drugs used for testing but at different levels (Fig. 1). Note that ***E. coli* is intrinsically resistant to penicillin**, therefore this antimicrobial is not used for testing of *E. coli* isolates.

When extrapolating from laboratory data to field cases from the information shown in Fig. 1, one should exercise caution. Laboratory data in general are biased in that they are often associated with clinical cases that may have undergone previous treatments. For that reason, **the level of resistance in our data may be higher than in the general population**. In addition, from the clinical perspective, our data are based on in vitro results. These results may or may not be reproduced in vivo.

Veterinarians are in the front-line in the war against antimicrobial resistance, and in maintaining the efficacy of antimicrobials, and as such **need to avoid the use of category I antimicrobials,** such as third (ceftiofur) and fourth generation cephalosporins, and fluoroquinolones (enrofloxacin), **and limit use of category II drugs**, e.g., aminoglycosides, the first and second generations of cephalosporins including cephamycins, penicillins, and trimethoprim/sulfamethox-azole - these 2 categories include most of the antimicrobials used in equine practice (see next page). **Serum amyloid A** is being used by many as a marker/decision factor in determining antimicrobial use in horses, and is included in all equine biochemical panels at

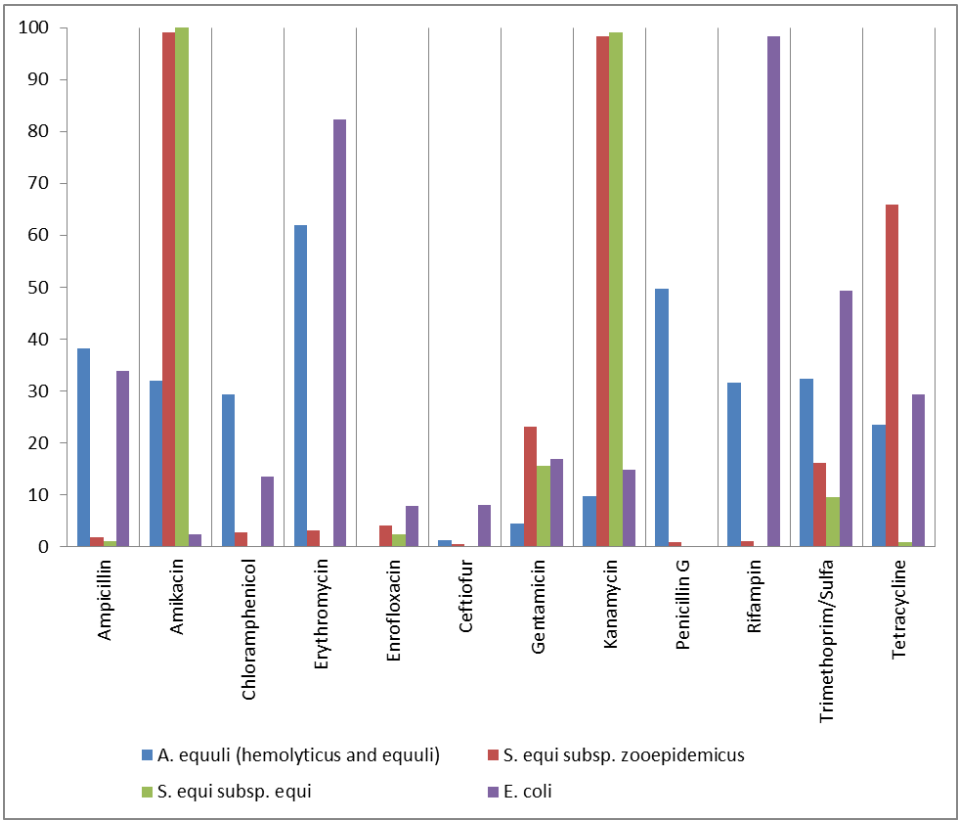

Figure 1. Antimicrobial resistance patterns of selected equine pathogens from 2007 to 2015. Data are shown as % resistant.

Continued - Antimicrobial susceptibility pattern of selected equine pathogens

Categories of antimicrobials used in equine practice, as they relate to human medical treatment.

Adapted from Health Canada's categorization of antimicrobials.

1. Category I: Very High Importance

These antimicrobials are considered of very high importance in human medicine as they meet the criteria of being essential for the treatment of serious bacterial infections and limited or no availability of alternative antimicrobials for effective treatment in case of emergence of resistance to these agents. Examples include:

- **Carbapenems**, e.g., imipenem
- **Cephalosporins,** third and fourth generations, e.g., ceftiofur
- **Fluoroquinolones,** e.g., ciprofloxacin, enrofloxacin
- **Glycopeptides,** e.g., vancomycin
- **Nitroimidazoles,** e.g., metronidazole
- **Penicillin-β-lactamase inhibitor combinations,** e.g. amoxicillin-clavulanic acid
- **Polymyxins** (colistin), e.g. polymyxin B
- Therapeutic agents for tuberculosis, e.g. rifampin

2. Category II: High Importance

Antimicrobials in this category consist of those that can be used to treat a variety of infections including serious infections and for which alternatives are generally available. Examples include:

- **Aminoglycosides** (except topical agents), e.g., gentamicin, amikacin, kanamycin
- **Cephalosporins**, first and second generations (including cephamycins), e.g., cefazolin
- **Macrolides,** e.g., clarithromycin, erythromycin, azithromycin
- **Penicillins,** e.g., penicillin, amoxicillin, ampicillin
- **Quinolones** (except fluoroquinolones), e.g., nalidixic acid
- **Trimethoprim/sulfamethoxazole** (TMS)

3. Category III: Medium Importance

Examples include:

- **Aminoglycosides**, topical agents
- **Bacitracins,** e.g., BNP eye ointment
- **Nitrofurans,** e.g., nitrofurazone
- **Phenicols,** e.g., chloramphenicol
- **Sulfonamides**
- **Tetracyclines,** e.g., doxycycline, oxytetracycline, minocycline
- **Trimethoprim**

4. Category IV: Low Importance

Antimicrobials in this category are currently not used in human medicine. These drugs are not frequently used in equine medicine either.

Reference Health Canada, Categorization of Antimicrobial Drugs Based on Importance in Human Medicine. http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr_ram_hum-med-rev-eng.php

Equine granulocytic anaplasmosis (*Anaplasma phagocytophilum)*

Kristiina Ruotsalo, Brent Hoff, Richard Ryan

A 17-year-old Appaloosa mare with a history of long-term oral prednisone treatment for severe recurrent airway obstruction (RAO), was presented to the referring veterinarian with a recent history of anorexia, depression, reluctance to move, and limb and ventral edema. Clinical evaluation revealed jaundice, pyrexia (40.5^{o}C), tachypnea, and tachycardia. A week previously, this mare had been treated for mild colic, which had resolved.

EDTA blood and serum were submitted to the AHL for a comprehensive CBC and biochemistry panel, and intravenous treatment with trimethoprim/sulfadiazine, flunixin, and Newcells was started while awaiting laboratory results.

The CBC demonstrated marked thrombocytopenia (platelet count 28 X10^{9} /L), and a mild left shift (0.40 X10^{9}/ L). **Examination of the peripheral blood smear revealed that ~35% of neutrophils contained intracytoplasmic morulae consistent with *Anaplasma phagocytophilum*** (Figures 1, 2). PCR subsequently confirmed the identity of these organisms as *A. phagocytophilum.*

Significant changes in the serum biochemistry profile included mild to moderate hypoproteinemia (total protein 48 g/L), a moderate increase in free bilirubin (142 µmol/L), and a marked increase in serum amyloid A (2,668 mg/L).

Following definitive identification of *A. phagocytophilum*, treatment was changed to intravenous oxytetracycline, administered once daily for 5 days. The mare responded well to treatment, and by completion of the antibiotic regime was noticeably less jaundiced with almost complete resolution of limb edema. A serum biochemistry profile and CBC taken 14 days from the date of presentation revealed a total serum protein of 58 g/L, free bilirubin of 5 µmol /L, and serum amyloid A of 0 mg/L. The platelet count was 175 X 10^{9}/L, no left shift was noted, and no morulae could be visualized within the neutrophils. *A. phagocytophilum* PCR was negative. The mare was clinically normal according to the owner.

A. phagocytophilum includes the organisms previously described as *Ehrlichia equi*, *Ehrlichia phagocytophila*, and the human granulocytic ehrlichiosis agent, to reflect close genome homology. ***A. phagocytophilum* is the causative agent of equine granulocytic anaplasmosis (EGA)**, and is found in membrane-bound vacuoles within the cytoplasm of infected neutrophils and eosinophils. These inclusion bodies consist of one or more coccoid or coccobacillary organisms ~0.2 µm in diameter, as well as large, granular aggregates (morulae), visible under light microscopy.

EGA typically occurs during late fall, winter, and spring. Horses of any age are susceptible, but clinical signs are less severe in horses <4 years of age. The disease is not contagious but thought to be transmitted by the western black-legged tick (*Ixodes pacificus*) in western North America, and **the deer tick (*Ixodes scapularis*) in eastern North America**. The pathogenesis of EGA is poorly understood, but after entering the dermis by tick-bite inoculation, the bacteria invade target cells of the hematopoietic and lymphoreticular systems. It is unclear if there is direct injury of infected cells, but localized inflammatory events are initiated within tissues containing infected cells. Peripheral sequestration, consumption, and destruction of peripheral blood components are all proposed as potential mechanisms of cytopenias.

Following an incubation period of ~10 days, infected horses may experience subclinical disease or develop overt clinical signs including fever, depression, anorexia, reluctance to move, limb edema, icterus, petechiae, and ataxia. Moderate to severe morbidity is seen occasionally with EGA, and occasional mortality has been reported. The disease is often self-limiting, and clinical signs usually last 7 -14 days. Anemia, variable leukopenia and thrombocytopenia are usually noted with clinical cases of EGA.

Diagnosis relies upon clinical awareness of geographic areas for infection, appropriate clinical signs and laboratory changes, and the presence of morulae within granulocytes in peripheral blood smears. As affected horses may be leukopenic, and because the number of granulocytes containing morulae can vary from 1% to 50% by day 3-5 of infection, **examination of a buffy coat smear can enhance detection of morulae**. PCR can confirm the clinical diagnosis and is particularly helpful in both the early and late stages of disease when the numbers of organisms may be too small for diagnosis by microscopy. **PCR testing for *A. phagocytophilum* is available at the AHL, and can be used for detection of the organism from whole blood, tissue, and ticks.**

A .phagocytophilum has not been reported previously in Ontario. Prevalence of this disease in Ontario is unknown, but **anaplasmosis should be considered as a differential**

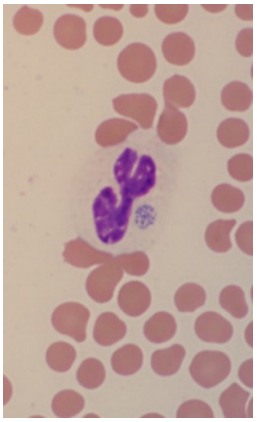

Figure 1. *A. phagocytophilum* morula in a

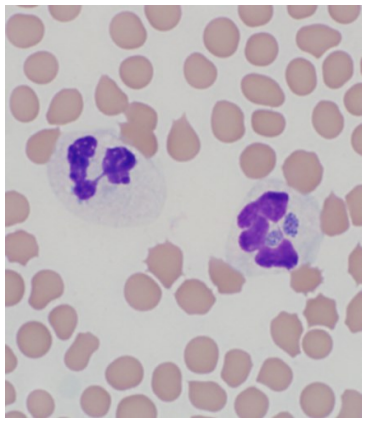

Figure 2. Two *A. phagocytophilum* morulae in a

COMPANION ANIMALS

NetF toxigenic *Clostridium perfringens* type A diarrhea in an 11-month-old dog *Iman Mehdizadeh Gohari, Durda Slavic*

A role for *C. perfringens* type A in hemorrhagic and necrotizing gastroenteritis of dogs has long been suspected but not well defined as this bacterium is also commonly present in the intestinal tract and feces of healthy animals. However, the recent discovery of a **novel beta-pore-forming toxin (NetF),** which is strongly associated with canine hemorrhagic gastroenteritis and foal necrotizing enteritis should improve our understanding of the role of *C. perfringens* type A in enteric disease. NetF has been shown to have cytotoxic effects on equine ovarian cells, and **it has been isolated from 8 of 11 (73%) cases of canine fatal hemorrhagic gastroenteritis**.

A fecal sample from an 11-month-old Labrador-cross dog with diarrhea of 4 days duration was submitted to the AHL for bacteriology testing. No ova were detected in the stool based on the fecal flotation test performed at the clinic. The sample was also negative for *Salmonella* spp., *Yersinia* spp., and *Campylobacter* spp., but large numbers of *C. perfringens* were isolated. Further characterization of *C. perfringens* revealed that was toxin type A and that it also carried a gene for NetF. No additional details on the clinical presentation and follow up were available.

Veterinarians should consider that NetF-producing *C. perfringens* type A strains may have a pathogenic potential in cases of canine enteritis, especially in hemorrhagic ones. *AHL*

Reference

Mehdizadeh Gohari I, et al. A novel pore-forming toxin in type A *Clostridium perfringens* is associated with both fatal canine hemorrhagic gastroenteritis and fatal foal necrotizing enterocolitis. PLoS One 2015 Apr 8;10(4):e0122684.

Plateletcrit *Kristiina Ruotsalo*

Canine CBCs now include an additional parameter generated by the Advia 2120 hematology analyzer, the plateletcrit (PCT). **The plateletcrit is derived from multiplication of the total platelet number by the mean platelet volume, and is an indicator of circulating platelet mass.** The plateletcrit can provide clinically important information when platelet numbers alone are an incomplete representation of primary hemostatic capacity, particularly in breeds of dogs with genetic macrothrombocytopenia such as Cavalier King Charles Spaniels, and Norfolk and Cairn Terriers. *AHL*

Lipase assay *Kristiina Ruotsalo*

The AHL Clinical Pathology Lab would like to reassure its clients that the **DGGR lipase assay**, which has recently gained exposure as a 'novel' test for the diagnosis of pancreatitis, has been a **standard test within our canine and feline biochemistry panels** for more than 10 years.

We continue to offer the DGGR-lipase assay for your canine and feline patients. *AHL*

Two new AHL LabNotes available

Thanks to Dr. Josepha DeLay and colleagues, we've posted 2 new LabNotes on the AHL website: http://www.guelphlabservices.com/AHL/LabNotes.aspx

AHL LabNote Number 41 November 2015

Fixation and transport of large excisional biopsies AHL Histology Laboratory

AHL LabNote Number 42 November 2015

Field and clinic postmortems: Simplified protocol and image list

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AHL Newsletters and LabNotes are available on the Web at - http://ahl.uoguelph.ca