UNIVERSITY of GUELPH
LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Dr. Brian Binnington retires!

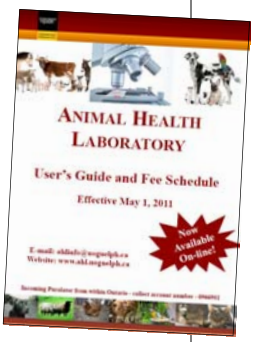
Brian is well-known and respected as an experienced veterinary pathologist with competence in all species and a keen interest in poultry. Brian is a 1973 OVC DVM graduate, with a Diploma in Veterinary Pathology (OVC 1974), as well as a Diplomate of the American College of Veterinary Pathologists (1986). Brian has taught at both WCVM and OVC, and has worked as a pathologist and lab head in Brighton, ON (VLSB 1977-1996), 2 years in private industry, in Guelph (AHL 1998-2006), and since 2006 at AHL-Kemptville.

Brian has contributed many years of service to the veterinary and animal-owning communities in Ontario and beyond, and we wish him a long and happy retirement (and much enjoyment from his new red truck!).

Dr. Andrew Brooks joins AHL-Kv

Dr. Andrew Brooks will be joining the AHL/Lab Services May 30, 2011 as our new **Veterinary Pathologist/Laboratory Head, Kemptville**. Andrew holds a BA from Queen's (1992), a DVM degree (OVC 1996) and a PhD (OVC 2003), and is a Diplomate of the American College of Veterinary Pathologists (2004). He has extensive diagnostic lab and teaching experience in both Canada and the USA, plus experience in both large and small animal practice, as well as in private industry. We are delighted to have Andrew join us in the AHL-Kemptville lab, and look forward to his contributions to our clients and our laboratory system.

May 1, 2011, AHL User's Guide and Fee Schedule



- Effective May 1, 2011. Mailed mid-April - we would be happy to send clients additional copies.
- View and search available tests at http://www.ahl.uoguelph.ca—click on Available Tests.
- To log in for fees, for Vet-clients only - at http://www.ahl.uoguelph.ca Contact Josie at AHL ext. 54320 or ahlinfo@uoguelph.ca for the **Client Access** username and password.
- Access to online results is also available – please contact us for further details ahlinfo@uoguelph.ca
- Updated pricing effective May 1, 2011 to April 30, 2012.
- Bacteriology testing update: **Bacterial culture, companion/other**, now includes susceptibility! (You still need to add setup fee so $20 +$20) - **Bacterial culture, follow-up, companion/other**, a follow-up test for previously cultured sample(s) within 2 months of initial submission. *AHL*

Potomac horse fever *Beverly McEwen*

Potomac horse fever (PHF), also known as **equine monocytic ehrlichiosis,** is caused by *Neorickettsia risticii*. Since May 2007, 21/60 (35%) horses have been positive for Potomac horse fever (PHF) with the tests requested. Positive horses were in southwest, central and eastern Ontario. Most commonly requested tests are the PHF indirect immunofluorescent antibody (IFA) test on serum, and the PHF PCR on EDTA blood from live animals, or lung, liver, spleen from post-mortem cases. *AHL*

Approved projects - AHSI 5th call for proposals (winter 2011)

Proj. ID	Project lead; Project team members	Project title	Project term
11-01	Michele Guerin, OVC; Gwen Zellen, Babak Sanei, Csaba Varga, Agnes Agunos, Dean Middleton	*Salmonella* Enteritidis baseline study in Ontario commercial broilers.	2 yr
11-02	Hugh Cai & Marina Brash AHL; Emily Martin, Margaret Stalker	Avian *Chlamydophila* and *Coxiella* infection in Ontario: molecular diagnosis method development.	2 yr
11-03	Paula Menzies, OVC; Sarah Wootton, Susy Carman, Beverly McEwen, Jocelyn Jansen	Detection of *Visna/maedi virus* infection in Ontario sheep flocks.	1.5 yr
11-04	Josepha DeLay, AHL; Murray Hazlett, Susan Lapos	Development of immunohistochemical tests for small ruminant lentiviruses (*Caprine arthritis-encephalitis virus*, *Visna/maedi virus*).	0.5 yr
11-05	Durda Slavic, AHL	Validation of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) for bacterial speciation.	1 yr
11-06	Durda Slavic, AHL; Davor Ojkic, Keith Harron, Beverly McEwen, Babak Sanei, Teresa Cereno, Edward Malek	Comparison of four different sample types and three different culture methods for *Salmonella* spp. detection in poultry environmental samples.	1 yr
11-07	Davor Ojkic, AHL; Sarah Hoyland, Elizabeth Hillyer	Evaluation of massively parallel sequencing for detection and characterization of viruses.	1 yr
11-08	Javier Sanchez AVC, UPEI; Zvonimir Poljak, Crawford Revie, Caroline Dube, Bruce McNab	Contact network based disease spread model in Ontario.	1 yr
11-09	Davor Ojkic, AHL; Michele Guerin, Michael Eregae, Thelma Martinez	Genotyping of fowl adenoviruses and infectious bursal disease viruses from Ontario broiler breeder flocks.	1.5 yr
11-10	Hugh Cai, AHL	Development of rapid identification assays for veterinary mycoplasmas using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.	1.5 yr
11-11	John Lumsden, OVC; Lincoln Tubbs, Spencer Russell	Diagnosis and surveillance of *Chlamydia*-like organisms in Ontario rainbow trout.	1.5 yr
11-12	David Kelton, OVC; Ann Godkin, Richard Cantin, Deb van de Water, Jim Fairles, Davor Ojkic, Durda Slavic	Equivalency for strong positive serum ELISA Johne's disease test results and interpretation of repeated testing for Johne's disease in Ontario dairy herds.	1.5 yr

AHL Newsletter
June, 2011 - Volume 15, Number 2
Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistant: **Helen Oliver, Amanda Budd**

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.

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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.

Trace mineral status in ruminant livestock – How to sample

Brent Hoff, Nick Schrier

Blood trace mineral concentrations are affected by multiple variability factors. Sampling adequate numbers of animals aids in reducing the effect of extraneous variation. Care should be taken to use the proper sampling protocol, in order to not cause artifacts that result in variation. **Removal of serum or plasma from the red cells within 2 hours of sample collection is an important step, as well as using the proper trace mineral sampling vial (dark blue top**, i.e., BD 368381). Plasma is easier to separate from red blood cells than serum, and it should be remembered that *serum should not be used* for copper analysis in cattle and sheep because ceruloplasmin is lost during clotting and copper values can be significantly lower in serum than plasma from paired blood samples. *AHL*

AHL proficiency testing program participation

Liz King

The Animal Health Laboratory (AHL) participates in proficiency testing programs to demonstrate the competency of our methods, equipment and staff. Proficiency testing (PT) is the use of inter-laboratory comparisons to determine the performance of individual laboratories for specific tests or measurements and to monitor a laboratory's performance. Participation in proficiency testing schemes provides laboratories with an objective means of assessing and demonstrating the reliability of the data they are producing.

By continually demonstrating competency, the AHL holds many accreditations as shown on our web page: http://www.guelphlabservices.com/AHL/About.aspx

The AHL participated in more than 46 different proficiency test programs in 2010/ 2011:

1. **Biological programs** – panels of samples are tested, or identification agrees with consensus ID and results are usually pass / fail. The AHL participates in the following biological PT programs: AAVLD bacteriology (IBQAS) • Boehringer-Ingelheim VetMedica PRRSV RT-PCR and PCV-2 PCR • CFIA (EIA ELISA) • CFIA National Centre for Foreign Animal Disease (AIV rtRT-PCR, NDV rtRT-PCR, CSFV RT-PCR, FMDV RT-PCR, FMDV ABC ELISA) • CFIA scrapie panel exchange • CFIA TSE panel • International proficiency testing scheme for Leptospirosis MAT • NVSL (*Salmonella* group D check test, coronavirus ring test) • Veterinary Laboratory Association - Quality Assurance Programs (VLA QAP) - parasitology and histopathology • UK-NEQAS veterinary immunocytochemistry • USDA Johne's proficiency • USDA APHIS (NVSL) PRRSV antibody ELISA and PRRSV IFA antibody.

2. **Chemical programs** which produce numerical results and the results are usually categorized as satisfactory, questionable, or unsatisfactory. The AHL participates in the following chemical PT programs: Association of American Feed Control Officials (AAFCO) feed • CALA soils • FAPAS (metals, DON, ZON in food/feed) • Minnesota Department of Agriculture Manure Analysis program (MAPP) • North American Proficiency testing program (soil and plants) • OMAFRA soil fertility and plant analysis • OFA thyroid program • Randox Clinical chemistry • Randox Coagulation • Siemens urine QA/QC • VLA-QAP clinical chemistry - chemistry, endocrinology, serology, and urinalysis • Wibby biosolids/sludges.

The AHL participates in this extensive biology and chemistry PT program in order to continually demonstrate the competency of our methods, equipment, and staff. This is our commitment as a full-service Animal Health Laboratory providing you with the services you need and results you can trust. *AHL*

Bartonella spp. PCR test validation

Hugh Cai, Ana Rita Rebelo

The AHL has completed analytical validation of a *Bartonella* spp. PCR for canine and feline blood samples, which was previously optimized and evaluated from published assays in Dr. Prescott's lab in the Department of Pathobiology. The AHL will offer this test for free from June 1 to July 31, 2011, thereafter for a fee of $32.

The assay is specific for the *Bartonella* genus. If necessary, species can be identified by sequencing of the PCR products at an additional charge. *AHL*

Reference

Kamrani A , et al. The prevalence of *Bartonella*, hemoplasma, and *Rickettsia felis* infections in domestic cats and in cat fleas in Ontario. Can J Vet Res 2008;72:411-419.

AHL Lab Reports

RUMINANTS

Early gestation abortions in Ontario cattle

Brian Binnington, Beverly McEwen, Jan Shapiro

Bovine fetuses and placentas that are aborted during the first half of pregnancy are often small, poorly preserved or mummified. To evaluate the questioned diagnostic value of these submissions, we reviewed abortion cases with fetuses that were less than 5 months of age or with a crown-rump length less than 40 cm. From Jan. 2000 to Dec. 2010, 91 cases (9%) fit these criteria out of 1,013 cases of bovine abortion in the AHL database; 68 cases (75%) were abortions with a diagnosis and 23 cases (25%) were classified as abortion with undetermined cause. Testing typically included histopathology, bacteria, ureaplasma and mycoplasma cultures, virus isolation, maternal *Neospora caninum* ELISA and *Leptospira* MAT serology. Maternal serology for *Bovine herpesvirus 1* (IBRV), *Bovine viral diarrhea virus* (BVDV-1 and -2) and immunohistochemistry (IHC) for IBRV, BVDV and *Leptospira* spp. were conducted on a few of the cases.

Of the 68 cases with a diagnosis, abortion with *Neospora*-like organism was diagnosed in 36 (53%) of the cases. **Neosporosis** was diagnosed by positive maternal *Neospora caninum* ELISA serology and/or histopathology with characteristic lesions. **Bacteria-associated abortion** characterized by placentitis, with or without systemic lesions and intralesional bacteria, was diagnosed in 16 (24%) of the cases. Various bacteria were isolated including *Escherichia* coli, *Streptococcus spp.*, *Listeria monocytogenes*, *Arcanobacterium pyogenes*, *Bacillus licheniformis*, *Salmonella* Kentucky, *Campylobacter fetus* subsp. *fetus* and *Enterobacter agglomerans. Ureaplasma diversum* was isolated in 1 case. Fungus (*Aspergillus fumigatus*) and yeast (*Candida parapsilosis)* were isolated in 1 and 2 cases respectively. Placentitis without an apparent cause was present in 6 cases. **Viral abortions** included IBRV identified by IHC (1 case) and BVDV-1 by isolation (2 cases) and BVDV-2 by seroconversion in the cow (1case). A 21-cm fetus had multiple skeletal, renal and brain anomalies of undetermined cause.

Six of the 23 idiopathic abortion cases were small (15-24 cm) decomposed and mummified fetuses. The other 17 cases of idiopathic abortion tended to be smaller fetuses (6 < 10 cm, 3 <20 cm and 8< 30 cm). **Meaningful information as to the cause of the abortion was identified in 75% of these cases with small and often poorly preserved aborted fetuses. The majority of the cases involved infectious agents, with neosporosis predominating**. *AHL*

Pestivirus infection in an aborted ovine fetus

Margaret Stalker, Susy Carman, Murray Hazlett

A 30 cm crown-rump length ovine fetus and placenta aborted by a ewe from a flock of approximately 100 cross-breed sheep were submitted to the AHL for necropsy. A previous submission of 2 aborted fetuses from this flock was diagnosed as abortion due to *Campylobacter fetus* ssp *fetus* infection, with concurrent placentitis due to *Toxoplasma* infection. Similarly, in the current case, large numbers of *C. fetus* ssp *fetus* were isolated from placenta, fetal lung and stomach content. Although multiple foci of necrosis were also evident on histology of placental cotyledons, *Toxoplasma* immunohistochemistry (IHC) was negative. However, **a noncytopathic *Bovine viral diarrhea virus* (BVDV)-like pestivirus was isolated in cell culture from the fetal tissues**, and confirmed using BVDV monoclonal antibodies in indirect fluorescent antibody assays, as well as by IHC, using an anti-BVDV monoclonal antibody.

Pestiviruses and their natural hosts include BVDV of cattle, *Border disease virus* (BDV) of sheep, and *Classical swine fever virus* (CSFV) of pigs. Host infection and *in utero* transmission of these viruses to the fetus can result in early embryonic death, abortion, congenital abnormalities, or lifelong persistent infections. BVDV and BDV are genomically and antigenically similar. Although they are normally isolated from their primary host species, they are capable of infecting other species. For example, BVDV has been reported to also infect pigs, alpacas, wild deer, elk, sheep and goats. Natural and experimental infections of sheep and goats by BVDV can result in outbreaks of reproductive loss and abortion.

Since the spring of 2009, the AHL Mammalian Virology laboratory has performed virus isolation on fetal tissues from 140 cases of ovine and caprine abortions, as part of a surveillance program under the AHSI Small Ruminant Abortion Project. This case is the first isolation of a BVDV/BDV virus in this project. Review of AHL Mammalian Virology case records dating back to 1988 revealed only 12 isolates of a noncytopathic BVDV-like virus from ovine and caprine submissions. Due to the close genomic similarity of BVDV and BDV, final determination of the identity of the isolate in this case was not possible without viral genome sequencing.

Continued - Page 13

Pestivirus ovine abortion, cont'd

The flock in question was housed in close proximity to cattle, suggesting that this fetal infection may have been a result of transmission of true BVDV from acutely or persistently-infected cattle on-site. Alternatively, transmission of BDV to this fetus may have been from a persistently infected sheep within the flock. Both viruses can be transmitted by oral, conjunctival, intranasal or genital routes. **Although unproven in this case, the potential for cross-species transmission of BVDV is a recognized risk of husbandry practices which allow contact or commingling of cattle and small ruminants.** *AHL*

Small ruminant abortion project ends

Hugh Cai, Murray Hazlett, Jim Fairles

This project officially ended Friday May 6, 2011. Submissions received after this date will be billed as routine cases. We would like to thank all of the producers and veterinarians who submitted animals and helped to generate a lot of useful information. Results of this intensive study will be appearing in future newsletters - stay tuned! *AHL*

SWINE

New *Rotavirus* PCR test improves diagnostic rate for porcine diarrhea

Susy Carman, Murray Hazlett, Beverly McEwen, Kurt Rossow

From 1998 until April 2011, using the *Rotavirus* latex agglutination (RLA) test alone, about 12% of swine diarrhea cases have been found to be positive for *Rotavirus A* (Figure 1). Since implementing the *Rotavirus A*/C rtRT-PCR and the *Rotavirus B* RT-PCR in 2010, originally validated by the University of Minnesota, the diagnosis of *Rotavirus*-associated diarrhea in pigs has markedly increased. From 2010 to April, 2011, 9/53 (17%) cases requesting RLA were positive, whereas, 19/28 (68%) cases requesting the *Rotavirus* group RT-PCR tests were positive. Of these, 8 were positive for *Rotavirus B*, 6 were positive for *Rotavirus A*, and there was 1 case each of dual infection with *Rotavirus A* and *B* and *Rotavirus A* and *C*. **The ability to identify *Rotavirus B* and *C* by RT-PCR has improved the ability of AHL pathologist to confirm *Rotavirus* infection, compared to RLA** (Figure 1).

Rotavirus was the only pathogen identified in 10 cases, 8 of which were tested for additional pathogens. Confirmation of concurrent infections depends upon the additional tests requested: 4 *Rotavirus* RT-PCR cases had concurrent *E. coli* infections, 2 were positive for *Salmonella* sp., 2 were positive for both *E. coli* and *Salmonella* sp, and 1was also positive for *Clostridium difficile*.

Rotaviruses infect the enterocytes of the tips and sides of villi. Similar to *Transmissible gastroenteritis virus* (TGEV), *Rotavirus* is most prevalent within enterocytes 24 hours after experimental infection, and after 4 days very little virus may be present. Syncytial cell formation is sometimes seen with *Rotavirus* (see photo, Sept 2010 Newsletter), and microvilli become irregular and shortened. The exfoliation of the infected cells results in villus atrophy (Fig 2), and the mucosal surface is covered by flattened immature epithelium that later matures but leaves behind shortened (atrophic) villi. *AHL*

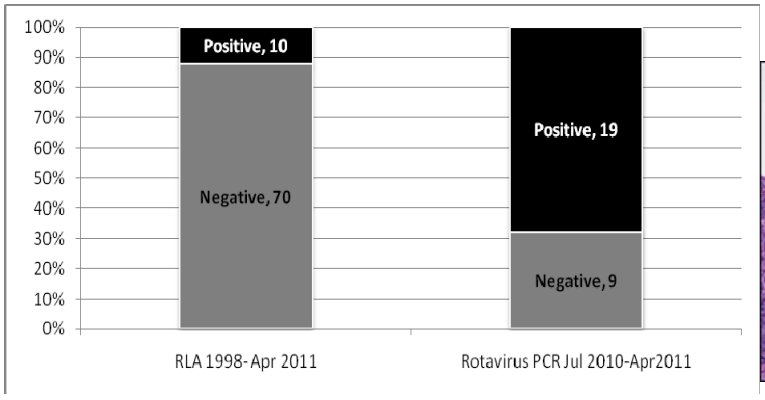

Figure 1. Comparison of the positive rate of *Rotavirus* latex agglutination and *Rotavirus A/C* rtRT-PCR and *Rotavirus B* RT-PCR.

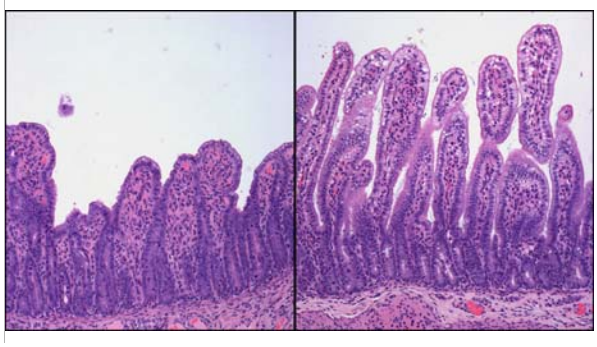

Figure 2. Villus atrophy typical of porcine *Rotavirus* or TGEV (left). Normal intestine on right.

AVIAN/FUR/EXOTIC SPECIES

Salmonellosis in pigeons

Marina Brash, Durda Slavic, Beverly McEwen

In early February, a racing pigeon loft composed of 90 birds experienced a spike in mortality that began with 2 cocks in good body condition dying suddenly with no clinical signs. Birds continued to die, 1 at a time, and in most cases the only clinical signs were those of reduced feed and water intake 24 hours prior to death. Some birds did linger for a week before dying, and those birds did lose weight. A 5-day treatment of amoxicillin via the water was instituted in mid-February and the mortality slowed for a week and then resumed. Three live birds were submitted for necropsy in mid-March, by which time the owner had lost over 40% of his birds. Two of the 3 birds were in good body condition but were not eating. One bird had mild multifocal hepatitis and focal laryngitis; the other bird had localized granulomatous pneumonia involving the right lung and osteomyelitis of the left mandible (Figures 1, 2). The third bird was in poor body condition, not eating and dehydrated, and had focal granulomatous pneumonia of the left lung, marked multifocal hepatitis, nephritis, pancreatitis and enteritis. *Salmonella* Typhimurium var Copenhagen was recovered from the multiple organs; this serotype is the most common type isolated from pigeons.

Further discussion with the loft owner revealed that he had received 2 birds from another pigeon fancier in the summer of 2010, and birds have been added directly into the new loft without an initial period of quarantine and no fecal swabbing for *Salmonella* culture. In February, all birds were medicated and the birds did respond for a short time but quickly relapsed. The temperatures in mid-February were very cold and pigeons typically dramatically reduce their water consumption, so it is likely they did not consume adequate amounts of antimicrobials, which would help explain the partial response to treatment followed by the quickly reappearing mortality. Following retreatment and thorough cleaning and disinfection of the loft, no more sick or dead birds have been reported.

The Animal Health Laboratory diagnoses of salmonellosis in pigeons were reviewed from 2005 to present, and although the annual number of salmonellosis diagnoses has remained relatively constant, the proportion of salmonellosis cases has increased substantially (Table 1). The relationship of this to the actual prevalence of salmonellosis in pigeons is not known, due to the submission biases to the AHL. We will continue to monitor this disease in pigeons in order to determine if this trend continues. **Pigeon owners need to be reminded that salmonellosis has public health significance as *Salmonella* is a zoonotic agent that can cause illness in people.** *AHL*

Table 1. Number and percentage of AHL salmonellosis diagnoses in pigeons, 2005 to 2011.

Year	2005	2006	2007	2008	2009	2010	Jan-Mar 2011
# *Salmonella*-positive cases	2	1	1	1	3	3	1
Total necropsy cases	22	41	33	41	16	14	3
% *Salmonella*-positive cases	9	2.4	3	2.4	18.7	21.4	33

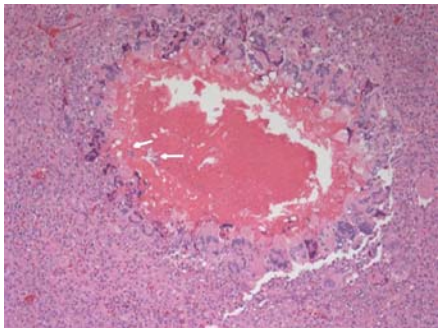

Figure 1. Granulomatous pneumonia, with bacterial colonies (arrows).

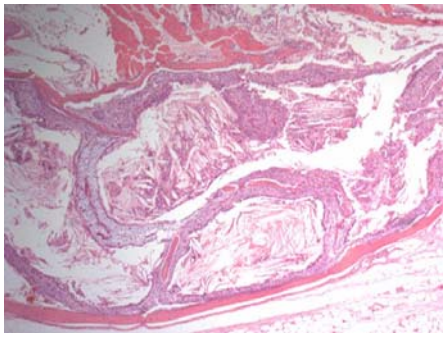

Figure 2. Osteomyelitis of the mandible.

HORSES

Nocardioform equine placentitis

Durda Slavic, Krishna Yekkala, Arthur Rotenberg, Tony van Dreumel

Recently, 2 cases of equine nocardioform placentitis, previously not reported in Ontario, have been confirmed by the Animal Health Laboratory.

Nocardioform equine placentitis was first described in Kentucky in the late 1980s, and over the years it became an established disease in that area. Only sporadic cases of nocardioform placentitis have been reported in other geographical regions including Florida, South Africa, and Italy. This type of placentitis is caused by gram-positive, filamentous branching bacterium belonging to the Actinomycete group. In this group, the 2 species most frequently isolated from cases of nocardioform placentitis are *Amycolatopsis* and *Crossiella* species. Both of the cases of nocardioform placentitis in Ontario were caused by *Amycolatopsis* spp.

Nocardioform placentitis often results in late-term abortion, stillbirth, or premature delivery. No vaginal discharge is associated with the disease, but there may be premature moderate mammary gland development. There may be premature separation of the chorioallantois resulting in a red-bag delivery. **The placentitis is usually located in the body of the uterus away from the cervix, and changes are found on the chorionic side of the placenta.** The affected area is usually covered with thick, sticky, brown, mucopurulent material. Most mares affected by nocardioform placentitis do not require subsequent treatment and do not have clinical signs of infertility the following breeding season.

Diagnosis of nocardioform placentitis is confirmed by bacterial culture and histological examination of placenta. Since nocardioform organisms are slow growing organisms, they require an extended incubation time, and bacteriology results are usually available in 5 to 10 days. *AHL*

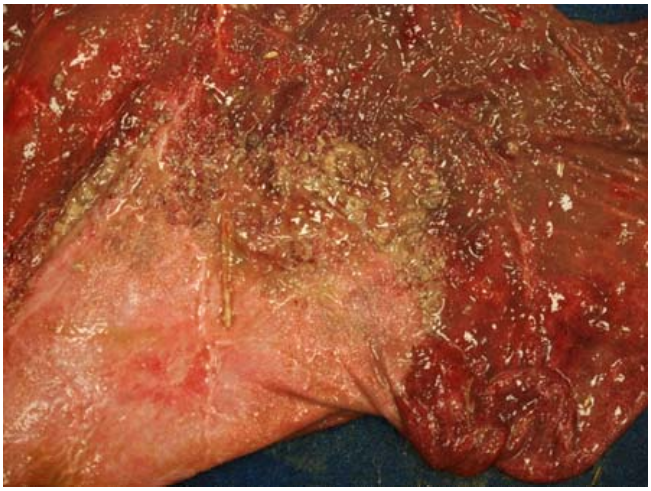

Figure 1. Mucopurulent exudate on the chorionic surface of a placenta in a case of equine nocardioform placentitis.

Equine herpesvirus update

Susy Carman, Beverly McEwen, Josepha DeLay, Murray Hazlett, Margaret Stalker

Equine herpesvirus (EHV) is a common virus of horses. EHV-1 causes respiratory disease, abortions, neurologic disease and is occasionally identified in weak neonatal foals. EHV-4 is usually associated with respiratory disease and may cause abortions. EHV is detected by identifying viral antigens with fluorescent antibody (FAT) or immunohistochemistry (IHC) and viral nucleic acid is detected with PCR.

The proportion of PCR/FAT/IHC-positive EHV cases has increased in the spring of 2011, compared to previous years (Figure 1). The majority of these cases are abortions (n=8) and a single case each of premature delivery of a weak neonate and encephalitis in a mature horse.

Since 2007, 3 neuropathogenic and 9 non-neuropathogenic strains of EHV-1 have been confirmed at the AHL, the most recent neuropathogenic case occurred in March 2011. *AHL*

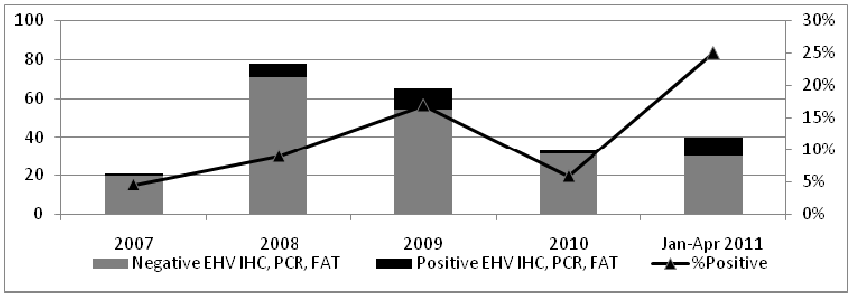

Figure 1. proportion of PCR/FAT/IHC-positive EHV cases at the AHL, 2007-2011.

COMPANION ANIMALS

Xylitol toxicity in a Golden Retriever *Kristiina Ruotsalo*

A 6-year-old Golden Retriever was presented to the referring veterinarian approximately 24 hours after consuming an unknown number of muffins prepared with xylitol. The dog was seizuring upon presentation and developed hematomas at venipuncture sites. CBC results revealed mild hemoconcentration, mild thrombocytopenia (platelet count 109 x 10^9/L), mild mature neutrophilia and lymphopenia. The serum biochemistry profile revealed numerous alterations in liver parameters including a profound increase in ALT 27,790 U/L (reference interval 19-107 U/L), a mild to moderate increase in ALP 499 U/L (22-143 U/L), moderate increases in both free bilirubin 25 µmol/L (0-3 µmol/L) and conjugated bilirubin 39 µmol/L (0-1 µmol/L), along with a mild increase in GGT 17 U/L (0-7 U/L). Serum bile acids were 560 µmol/L (0-6 µmol/L). Serum glucose was decreased at 2.2 mmol/L (3.3-7.3 mmol/L), as were serum potassium 3.1 mmol/L (3.8-5.4 mmol/L) and chloride 93 mmol/L (104-119mmol/L). Mild azotemia was noted. Prothrombin time and partial thromboplastin time were markedly increased at > 100 seconds. Due to the poor clinical prognosis, the dog was euthanized.

Xylitol is a 5-carbon sugar alcohol which occurs naturally in low levels in fruits and vegetables. It is being increasingly used as a sugar substitute in chewing gum, candy, nicotine gum, toothpastes, baked goods, and is also available in a granulated format for home cooking. The increased popularity of xylitol is based on the fact that it is as sweet as sucrose but contains only two-thirds the calories of sugars. It causes little insulin release in humans, and is thus suitable for low-carbohydrate and low-glycemic-index diets. Xylitol has been shown to inhibit oral bacterial growth, thus exhibiting anticariogenic properties.

Once thought to only induce hypoglycemia in dogs, xylitol can also produce life threatening acute hepatic necrosis. Following ingestion, vomiting is typically noted. Hypoglycemia may then develop within 10 to 60 minutes due to xylitol's potent insulin stimulatory effects. Delayed hypoglycemia may occur with some cases of xylitol gum ingestion. Clinical signs may then progress rapidly from lethargy to ataxia, collapse and seizure activity. **Xylitol doses >0.1 g/kg may cause hypoglycemia, and doses of >0.5 g/kg may result in hepatotoxicity**. **It is unclear whether hepatic failure is truly a dose dependent or idiosyncratic reaction.** Hepatic damage may be noted as soon as 9-12 hours post-ingestion, or be delayed up to 72 hours. Hepatic necrosis may result in exacerbation of hypoglycemia and secondary disseminated intravascular coagulation. Depression, vomiting, icterus, melena, diarrhea, petechiation/ecchymosis, and hepatic encephalopathy may be noted with clinical disease progression.

It is imperative to educate dog owners about the potential consequences of xylitol consumption, and to raise awareness of the increased use of xylitol within foodstuffs. *AHL*

Cocoa mulch and dogs (theobromine poisoning) *Brent Hoff, Nick Schrier*

Cocoa bean shells are frequently used in horticulture as mulch. Cocoa mulch, which is sold by most garden supply stores, contains large amounts of theobromine and caffeine, which are toxic to pets. Due to their indiscriminate eating habits, dogs of any age or breed can be affected by ingesting this material. Cats are less likely to eat the material, because they are not able to sense the sweet taste.

Theobromines are methylated xanthine alkaloids (methylxanthines) that cause a variety of clinical signs, including vomiting, diarrhea, PU/PD, ataxia, cardiac arrhythmias, CNS stimulation and potentially death. Dogs are the most frequently intoxicated species, but cats, pigs, horses, birds and other animals can also be affected.

Domestic animals metabolize theobromine much more slowly than humans. Dogs and other animals can easily consume enough of the mulch to cause serious poisoning.

Theobromine is slowly absorbed, reaching peak plasma levels in approximately 10 hours. It is metabolized within the liver and has a half- life of approximately 17 hours, so in severe cases, **clinical signs of theobromine poisoning can persist for 72 hours**. The LD_{50} of theobromine in dogs is 140 mg/kg. Cocoa beans contain approximately 1.2% theobromine by weight and a handful of this material could cause bradycardia or tachyarrhythmia and possibly death of a pet.

Chemists with the USDA are investigating the use of theobromine as a toxicant to control coyotes that prey on livestock. *AHL*

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