

Animal Health Laboratory



AHL Newsletter

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Figure 1. Serum samples with progressively increased hemolysis.

December, 2011



tember 12, 2011. Kris comes to us with experience in both the animal health and the human health fields.

Welcome Kris!

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Kris

began in





Holiday hours, 2011: Guelph drop box and refrigerator are available 24/7 for specimen drop off.

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Full service 0900-1700		
0900 -1700*		
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0900 -1700*		
Limited testing services		
⁻ 0900-1700, in both - Guelph and Kemptville		
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0900 -1700*		
0900 -1700*		
Normal services resume		

* Services limited to Specimen Reception, emergency necropsies and bacteriology set-up in Guelph.

Effects of hemolysis on results for trace elements in

Serum Nick Schrier To demonstrate the effect that sample hemolysis has on the results of the trace element screen, serum samples were produced with increasing levels of hemolysis from 1 bovine blood sample (Figure 1). The samples were analyzed using the trace element serum panel (AHL test code *icpse*); results in Table 1. Results for manganese, cobalt and molybdenum showed no significant difference between samples. Iron showed the largest increase with increasing sample hemolysis. Zinc showed a 10% increase from sample A to samples B,C,D, E. In the worst case (sample F) the overestimates of the levels of copper, zinc and selenium were in the range of 20-30%. Cases of extreme sample hemolysis (Sample F) are noted on your report, but even slight sample hemolysis can have an effect on some results. AHL

Table 1. Results of analysis of bovine serum samples by the trace element screen.

Sample ID	Manganese (ppb)	Iron (ppm)	Cobalt (ppb)	Copper (ppm)	Zinc (ppm)	Selenium (ppm)	Molybdenum (ppb)
А	1.19	1.80	1.81	0.715	0.647	0.081	64.7
В	1.38	2.98	1.72	0.778	0.693	0.077	64.1
С	2.53	4.61	2.29	0.760	0.717	0.078	65.1
D	2.08	5.34	2.01	0.742	0.724	0.079	63.7
Е	1.71	14.7	1.96	0.716	0.723	0.078	61.0
F	1.86	53.9	2.05	0.864	0.972	0.102	64.0

New tests offered at the AHL, November 15, 2011

Hugh Cai, Ana Rita Rebelo, Hamid Haghighi, Murray Hazlett, Beverly McEwen, Jim Fairles

IDEXX Mycoplasma hyopneumoniae ELISA

In addition to our current Dako (Oxoid) *M. hyopneumoniae* ELISA, the AHL will offer the IDEXX *M. hyopneumoniae* ELISA as of November 15, 2011. Previously, some clients requested that Dako suspicious or positive samples be retested with the IDEXX ELISA. Now this can be done in-house. The charge for either ELISA is the same. **Please indicate on the submission sheet if you want to retest Dako ELISA suspicious and positive samples with the IDEXX ELISA.**

It is important to note that the 2 ELISAs are not identical in their performance. It has been reported that the Dako *M. hyopneumoniae* ELISA had better sensitivity than the IDEXX *M. hyopneumoniae* ELISA, and that the 2 ELISAs had 80% overall agreement. Similarly, we found that the 2 ELISAs had 74 % overall agreement on field serum samples. We continue to recommend that the Dako ELISA be used as a screening test.

Bovine papillomavirus PCR for diagnosis of equine sarcoids

As of November 15, 2011, the AHL is offering *Bovine papillomavirus* (BPV) PCR for the diagnosis of equine sarcoids. Equine sarcoids, attributed to infection with BPV-1 or -2, are the most commonly diagnosed cutaneous neoplasm in horses. Histologically, it can be difficult to differentiate sarcoids from schwannomas. **Sarcoids and schwannomas can be differentiated by BPV PCR as well as by immunohistochemistry (IHC) testing**, sarcoids being BPV PCR-positive and schwannomas being IHC S-100 protein-positive. In a recent small study at the AHL using paraffin-embedded histology specimens, all 15 tissue samples diagnosed as sarcoid histologically were positive for BPV PCR. All 14 non-sarcoid tissue samples were negative for BPV PCR.

Please submit fresh or paraffin-embedded tissue to the AHL Molecular Biology Section. The price for the PCR test is \$32.00 for fresh tissue and \$43 for paraffin-embedded tissue. Immunohistochemistry for S-100 is \$44.50 in addition to regular histology fees. *AHL*

AHL Newsletter

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Editor: Grant Maxie, DVM, PhD, Diplomate ACVP Editorial Assistants: Helen Oliver, Amanda Budd

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Mailing address & contact information:

(please return all undeliverable Canadian addresses to:) Animal Health Laboratory Laboratory Services Division, University of Guelph Box 3612, Guelph, Ontario, Canada N1H 6R8 Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072 Email: <u>holiver@uoguelph.ca</u>

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Contributors to this issue:

From Laboratory Services / Animal Health Laboratory: Marina Brash, DVM, DVSc, Diplomate ACVP Hugh Cai, DVM, MSc, DVSc Josepha DeLay, DVM, DVSc, Diplomate ACVP Jim Fairles, DVM, MBA **Phyllis Few** Patsy Graham, DipEnvEng Hamid Haghighi, MSc Murray Hazlett, DVM, DVSc, Diplomate ACVP Brent Hoff, DVM, DVSc DipTox Sarah Hoyland, MSc Mary Lake, AHT Emily Martin, DVM, MSc, Diplomate ACPV Beverly McEwen, DVM, MSc, PhD, Diplomate ACVP Davor Ojkic, DVM, PhD Ana Rita Rebelo, MSc Nick Schrier, MSc Durda Slavic, DVM, PhD Maria Spinato, DVM, DVSc, Diplomate ACVP Margaret Stalker, DVM, PhD, Diplomate ACVP Other contributors: Beth Hanselman, DVM DVSc, Mississauga, ON Andrew MacLeod, DVM, Caledon, ON Paula Menzies, DVM MPVM; Terri O'Sullivan, DVM PhD, Population Medicine; Melanie Ammersbach, DVM, Andrew Peregrine, BVM PhD DVM, Pathobiology, OVC Scott Reid, DVM, Dunnville, ON 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.

Antimicrobial susceptibility of *Clostridium perfringens* isolates of bovine, chicken, porcine, and turkey origin from Ontario.

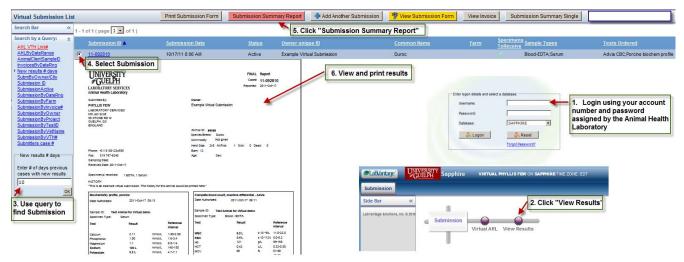
Durda Slavic, Patrick Boerlin, Martha Fabri, Kim Klotins, et al. (From Can J Vet Res 2011;75:89-97)

Antimicrobial susceptibilities and toxin types were determined for 275 *Clostridium perfringens* isolates collected in Ontario in the spring of 2005. Minimum inhibitory concentrations (MICs) of *C. perfringens* isolates for 12 antimicrobials used in therapy, prophylaxis, and/or growth promotion of cattle (n = 40), swine (n = 75), turkeys (n = 50), and chickens (n = 100) were determined using the microbroth dilution method. Statistical analyses and MIC distributions showed reduced susceptibility to bacitracin, clindamycin, erythromycin, florfenicol, and tetracycline for some isolates. Reduced susceptibility to bacitracin was iden-

tified in chicken (64%) and turkey (60%) isolates. Swine isolates had predominantly reduced susceptibility to clindamycin (28%) and erythromycin (31%), whereas bovine isolates had reduced susceptibility to clindamycin (10%) and florfenicol (10%). Reduced susceptibility to tetracycline was spread across all species. No clear reduced susceptibility, but elevated MIC(50) for virginiamycin, was found in chicken isolates in comparison with isolates from other species. Toxin typing revealed that *C. perfringens* type A is the dominant toxin type isolated in this study across all 4 host species. *AHL*

Animal Health Laboratory information at your finger tips *Phyllis Few*

6 simple steps to view your test results "on line", as soon as they are available!



Also

View the status of your submissions, the tests ordered, specimens submitted, copies of invoices, submission forms, link to Fee Schedule, Submission Forms and submittinginformation.

Access can also be given to log submissions remotely. AHL

Want to go paperless?

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- We'll provide you with a **username** and **password**.
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If you would prefer to receive an **electronic copy of the Newsletter** rather than a printed copy, please send your email address to us at <u>holiver@uoguelph.ca</u> The Newsletter is available on-line at <u>http://</u>

ahl.uoguelph.ca under "Publications".

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AHL Lab Reports **RUMINANTS**

Acute onset of illness in all ruminants in a zoo

Brent Hoff, Scott Reid, Patsy Graham

Zoo staff called the clinic to report that all of the giraffes, hippos, Malayan tapirs, and reindeer were lethargic and several animals had loose manure. The animals would not eat the ruminant alfalfa mix and they would not drink their water. The two giraffes would only browse on leaves. The feed was a 16% herbivore ration. A sample from one of the giraffes was sent to the AHL for fecal culture and fecal flotation and a feed sample was sent for mycotoxin analysis. The differential diagnosis at this stage was toxicosis due to nitrate/nitrite, lead, arsenic, T-2 mycotoxin, or feed additives.

All feed was withdrawn and replaced with new feed. No blood samples were available due to the difficulty of obtaining samples from these animals. The reindeer began to eat right away. The hippos ate the next day, and the tapirs and giraffes ate after several days.

Bacterial culture of the manure was negative for Salmonella. A mycotoxin screen of the feed was negative except for 0.5 ppm DON, which was clinically not significant. A feed additive screen was negative for narasin and salinomycin, but positive for monensin at 410 ppm. No monensin was supposed to have been present in the feed.

Feed refusal strongly suggested that something was wrong with the feed. The clinical signs, laboratory findings and the number of animals involved distinguished the case as a feed toxicosis rather than a deficiency. If the feed had not been removed quickly, clinical signs of cardiovascular collapse, weakness, decreased exercise tolerance, cyanosis, recumbency and death, may have been seen in affected animals. Toxic levels of monensin in cattle and sheep are 5 to 20 times those usually prescribed. Toxicity is usually associated with improper measurement or mixing of the drug into the feed. AHL

Fecal flotations revealed no evidence of parasites.

Hypovitaminosis E and sarcocystosis causing catastrophic losses in a

lamb crop Murray Hazlett, Paula Menzies, Nick Schrier

A producer of mixed-breed meat sheep submitted 7 lambs to the AHL for necropsy and workup after suffering 100 deaths out of 300 lambs from the spring lambing, housed 1 lamb, undetectable (<1.0 µmol /L) in 2 lambs. Ionophores indoors. These losses occurred over a 30-day period.

The affected lambs were housed with ewes - all yearling ewes lambing for the first time - in a new barn which was separated by partitions into 2 pens. Lambs losses started a few weeks prior to weaning but escalated postweaning. Only one pen was affected, and the ewes from this pen were also reported to be emaciated and had wool-slip. The other pen contained older weaned lambs that were not affected. The affected lambs had access to a commercial 18% starter ration and there was selenium in the ewe mineral at 60 ppm. The owner reported that the lambs were eating the starter well with estimated intakes of up to 1 kg/day.

At necropsy, all 7 lambs were extremely thin with marked subcutaneous edema; there was no serous atrophy of fat or hepatic lipidosis. Three had pneumonia associated with Mannheimia haemolytica, and all had a mild to moderate coccidial load. Histologically, aside from the pneumonia, the most striking finding were severe muscle degeneration with no significant mineralization and rare examples of active necrosis, and with very large number of Sarcocystis spp. cysts in skeletal and heart muscle and also tongue from 2 lambs. Selenium levels were low-normal on livers

from 3 lambs, and marginally low on serum from 1 lamb. Serum vitamin E levels were low: 1.0 µmol/L (ref 3.5-7.0) in were not detected in rumen content.

Wasting associated with vitamin E deficiency has been previously recognized in Ontario lambs. Why only 1 pen was affected is uncertain, and it is possible that dogs were defecating in this pen only, creating a large Sarcocystis spp. load in both the ewes and the lambs. Diet was different for the older lambs. The owner did note that at lambing, the dogs did spend time in the barn with this group of sheep. From time of infection to development of clinical signs is estimated to be 35 days. The poor condition of the ewes, in combination with the hypovitaminosis E and sarcocystosis in the lambs, likely caused the high lamb mortality.

Producers and veterinarians should be aware that vitamin E levels in feeds can decrease with storage, or may have been inadequate in prepared feeds to begin with. NRC recommendations for vitamin E were recently revised, with a recommended daily dose of 200 IU/day for a 20 kg lamb and 250 to 600 IU/day for lactating yearling ewes raising twins. Hypovitaminosis E should be on the list differentials for wasting in lambs and, because of the debilitated condition of the lambs, would be expected to be seen with other disease processes such as pneumonia. AHL

SWINE

Update on porcine gastritis and PFTS Josepha DeLay, Terri O'Sullivan

AHL pathologists and microbiologists are collaborating with Drs. Bob Friendship and Terri O'Sullivan, OVC Department of Population Medicine, to investigate the clinical significance and potential cause(s) of gastritis in weaned pigs, and **possible association between gastritis and post-weaning failure-to-thrive syndrome (PFTS).** We are currently contacting veterinarians who have previously submitted gastritis-positive animals for inclusion in a retrospective study. We are also recruiting herds with PFTS-type clinical signs for inclusion in a prospective study.

An educational video describing these clinical signs can be found at: <u>www.uoguelph.ca/~tosulliv/videofile/</u> (<u>user name</u>: guest1 / <u>password</u>: user1). Below the video is a link to a survey designed to determine the prevalence of PFTS in North America – your participation in this survey is requested and would be appreciated. For further information regarding participation in this project or to enroll a herd in the prospective study, please contact Dr. Terri O'Sullivan (519-824-4120, ext. 54079, <u>tosulliv@uoguelph.ca</u>) or Dr. Josepha DeLay (519-824-4120, ext. 54576, <u>jdelay@uoguelph.ca</u>). *AHL*



Figure 1. Gaunt appearance of typical PFTS-affected pigs.

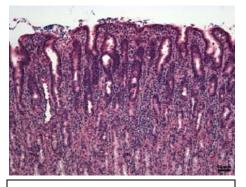


Figure 2. Erosive and lymphocytic gastritis in weaned pigs.

Re-emergence of selected parasites in swine

Maria Spinato, Josepha DeLay, Beverly McEwen, Andrew Peregrine

AHL pathologists have noted a recent increase in submissions related to downgraded hog carcasses and liver condemnations at slaughter. Common diagnoses include sarcoptic mange dermatitis and interstitial hepatitis or "milk-spotted" liver due to ascarid migration. Similar parasitic lesions, in addition to *Trichuris suis* colitis, have been diagnosed in unthrifty or diarrheic pigs submitted directly to the AHL by swine producers, some of whom selfidentify as organic livestock operations.

The goals of organic farming are to rear livestock under more natural conditions which include outdoor access where feasible, decreased indoor stocking density, foragebased diets, and reduced use of chemoprophylactic medications such as anthelmintics. In Europe, where organic livestock production systems have increased dramatically in recent years, these management changes have been associated with the re-emergence of both endoparasitic and ectoparasitic infections. **Sarcoptic mange has proven to be particularly difficult to control without the use of parasiticides;** severe infestations reduce growth rate and feed efficiency, increase culling and also represent an animal wel-

AHL pathologists have noted a recent increase in fare issue associated with distress due to pruritus.

Ascaris suum is also considered to have a welfare impact on some organic farms. Current research programs in Europe focus on management, dietary and alternative therapeutic strategies that will reduce the level of parasitic infections on organic farms.

Anecdotal information from veterinarians in Ontario indicates that there may be a similar increase in parasitic infections in organically raised pigs. In this province, limited use of parasiticidal drugs is permitted on organic farms if diagnostic data confirm a significant parasitic infection.

Additional epidemiologic studies are needed of Ontario swine farms to evaluate the risks and benefits associated with different productions systems, including organic operations. *AHL*

Reference

Thamsborg SM, Roepstorff A. Parasite problems in organic livestock production systems and options for control. J Parasitol 2003;89 (Suppl.):277-284.

AVIAN/FUR/EXOTIC SPECIES

An unusual case of Enterococcus cecorum septicemia in a racing

pigeon Marina Brash, Durda Slavic

In late March, the owner of a racing pigeon loft composed of 50 birds found a cock dead on a nest containing 2 7-day-old babies. The cock and 1 live youngster were submitted for necropsy. The adult pigeon was in good body condition and the crop was filled with grains and crop milk. The gizzard contained a small amount of grit and fibrous material, and the koilin was stained green-yellow. There was very little content in the small intestine. The liver was enlarged and congested and the spleen was small with congestion of 1 pole. The lungs were congested and edematous. There were no abnormal findings in the nestling.

Histologically, in the adult, the spleen was moderately congested, with moderate to marked lymphoid depletion and marked acute multifocal fibrinous necrosis and vasculitis with chains and pairs of small gram-positive coccoid bacteria both free and within macrophages (Figures 1 & 2). Other tissues including lung, liver, kidney, proventriculus, small intestine, brain, testis and skeletal muscle were congested and blood vessels contained numerous bacteria both free and within macrophages. Small clusters of bacteria were in myocardial capillaries and there was mild acute myocardial degeneration. The youngster had no significant histological findings.

Large numbers of *Enterococcus cecorum* were recovered from the lung and liver of the adult and from the crop content of the youngster. Verification of the bacterial identity was carried out using 16S rRNA partial sequencing.

Domestic pigeons are reported to carry *Enterococcus cecorum* in their crops, but to the best of our knowledge **this is the first report of** *E. cecorum* **causing septicemia in racing pigeons**. No other unusual mortality was reported in this loft indicating this may have been an opportunistic infec-

tion in an immunocompromised cock caring for young sters. $_{\mbox{\scriptsize AHL}}$

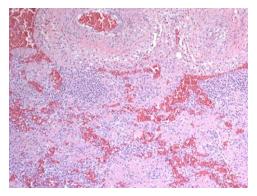


Figure 1. Moderate splenic congestion, lymphoid depletion, fibrinous necrosis and vasculitis with large numbers of bacteria.

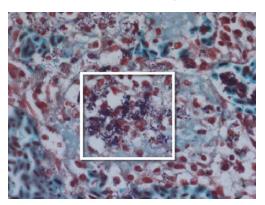


Figure 2. Pairs and chains of gram-positive cocci within a focus of fibrinous splenic necrosis.

Diagnosis of infectious laryngotracheitis at the AHL – update on available testing Emily Martin, Sarah Hoyland, Davor Ojkic, Marina Brash

The AHL has evaluated and implemented a PCR test, originally developed at University of Georgia, for quick identification of Infectious laryngotracheitis virus (ILTV, *Gallid herpesvirus 1*). This is a **real-time PCR test** with an estimated analytical sensitivity of 97.05% and specificity of 100%. Our recommendations for ILT diagnosis is for veterinarians to **submit both formalin-fixed tissue (eyelid, larynx/trachea, lung) and either swabs (up to 5 birds pooled) or fresh tissue (eyelid, trachea, lung, up to 5 birds pooled). If the fixed tissues arrive at the AHL before 1600 h, we can do a preliminary trim of even partially fixed tissue and then examine the histology slides the following after-**

noon to look for the herpesvirus inclusion bodies. In some cases we cannot identify these inclusions on histology, so we recommend running the new ILTV real time PCR as a concurrent test to compliment the histopathology testing. This will ensure the best chance for a quick diagnosis.

The ILTV real-time PCR is a detection assay and does not determine the virus strain. Followup testing involving virus isolation and virus typing by conventional PCR and sequencing can be done at an additional charge. This testing may take several weeks since the virus needs to be isolated before typing can be attempted. *AHL*

HORSES

West Nile and eastern equine encephalitis in horses in Ontario, 2008-

2011 Beverly McEwen, Davor Ojkic, Josepha DeLay, Murray Hazlett, Margaret Stalker

West Nile virus (WNV) and Eastern equine encephalitis virus (EEEV) are mosquito-borne pathogens able to cause disease in horses, people, deer, dogs and in some avian species. Clinical signs may include progressive weakness, depression, fever, muscle fasciculations, seizures, recumbency, ataxia and hyperesthesia. The case fatality rate for both viral infections is relatively high: 35-44% for WNV and 40-80% for EEEV.

- In live horses, IgM ELISA and virus/serum neutralization tests can be used for both viruses.
- At necropsy, specimens of brain and cranial spinal cord are submitted for real-time RT-PCR tests for EEEV and WNV following a negative *Rabies virus* test.
- Additionally, immunohistochemistry for WNV is available for formalin-fixed specimens.

Table 1. Number of cases tested for EEEV and WNV by IgM ELISA, virus neutralization, IHC and/or PCR, AHL, 2008-2011.

Year	EEEV positive/ tested	WNV positive/ tested
2008	0/16	1/20
2009	2/16	2/22
2010	2/13	1/16
Jan-Oct 14, 2011	3/15	7/20

In 2011, 8 WNV cases were identified in Grey (1), Simcoe (2) counties, Brantford (1) and Toronto (3); 3 EEEV cases were identified in Leeds and Grenville (1), Stormont, Dundas and Glengarry (2) counties. Data are regularly updated and mapped on the OMAFRA website: <u>http://</u> www.omafra.gov.on.ca/english/livestock/horses/facts/ <u>nhd_surv2011.htm</u>.

Vaccination history was provided for only 3 horses, none of which was vaccinated for WNV or EEEV. *AHL*

Verminous meningoencephalitis due to infection by Halicephalobus gingivalis in a Percheron gelding Margaret Stalker, Andrew MacLeod

A 7-year-old Percheron gelding was submitted to the AHL for necropsy following a brief clinical illness. The horse had been fed at 0900 h but had not finished his feed. At 2100 h, he was found to be recumbent, febrile (T=41 C) with an elevated heart rate (60 bpm), weak and minimally responsive, with nystagmus and apparent unawareness of his surroundings. The gelding was euthanized and sent to the AHL for necropsy. Differential diagnoses considered at the time of necropsy included viral neurologic diseases (*Rabies virus*, *West Nile virus*, EEEV, or EHV-1 infection), colic or acute gastrointestinal accident with terminal endotoxemia, acute toxicosis (e.g., ionophore toxicosis or ingestion of Japanese yew), head trauma, or hepatic encephalopathy.

The gross necropsy was unremarkable. Samples of brain sent for *Rabies virus* testing and EHV-1 PCR were negative. Upon further sectioning of the formalin fixed brain, areas of yellow discoloration and malacia were present, extending through the midbrain, hippocampus, thalamus and caudate nucleus. On histologic examination, these corresponded to areas of lymphoplasmacytic and histiocytic encephalitis centered on large numbers of small adult and larval nematodes, often within perivascular spaces or free within the neuroparenchyma (Figure 1, see p. 32). These nematodes were identified as *Halicephalobus gingivalis* (formerly known as *Micronema deletrix*) based on light microscopic characteristics.

Halicephalobus gingivalis is a ubiquitous freeliving saprophytic soil nematode, capable of causing sporadic opportunistic infections in horses and humans, presumably gaining entry through contaminated skin wounds or mucous membranes. In the horse, localized granulomatous infections of the oral cavity, eye or prepuce are reported, however, systemic dissemination, likely via hematogenous or lymphatic routes, may lead to infection of regional lymph nodes, lung, kidney, bone and/or, as in this case, brain.

Systemic infection, particularly of the brain, has a high fatality rate, although localized disease has in a few cases been amenable to surgical excision or ivermectin treatment. In the current case, no other site of infection was identified. Four human cases have also been reported in the literature; all developed fatal encephalitis, again possibly associated with entry and dissemination via contaminated skin wounds. *AHL* (Continued on p.32)

References

- Hermosilla C, et al. Fatal equine meningoencephalitis in the United Kingdom caused by the panagrolaimid nematode *Halicephalobus gingivalis*: Case report and review of the literature. Equine Vet J 2011;43:759-763.
- Sponseller BT, et al. Pathology in Practice. J Am Vet Med Assoc 2011;238:1265-1267.

Halicephalobus gingivalis in a horse Continued from p. 31

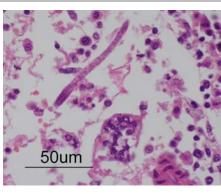


Figure 1: Histology of the brain demonstrating larval *Halicephalobus gingivalis* nematode adjacent to a multinucleated giant cell. (H&E).

Neorickettsia risticii (Potomac horse fever) testing Josepha DeLay

Please be aware that **EDTA blood samples** (2 mL minimum) are most appropriate for PCR testing for *Neorickettsia risticii*, the causative agent of Potomac horse fever. Although testing is sometimes requested using tissue and fecal samples, technical issues with these samples can have a significant negative impact on the sensitivity of the assay. For horses submitted for ne-cropsy with a history of enterocolitis, please include a pre-mortem EDTA blood sample with the submission in order to ensure appropriate sample availability for PHF testing, if necessary. *AHL*

COMPANION ANIMALS

Lung flukes (Paragonimus kellicotti) in a Labrador retriever

Brent Hoff, Beth Hanselman, Mary Lake, Melanie Ammersbach

A 5-year old neutered male Labrador retriever developed repeated episodes of coughing with blood in the sputum, over a 2- week period. The dog had a similar episode last year and improved after treatment ("white powder for parasites"). The dog had mild dyspnea and occasional inspiratory crackles on the left side, with mild sensitivity to tracheal palpation. He had a normal energy level with no changes in his physical condition or appetite. He had a history of UTI in the spring after eating some turtle eggs; this resolved with antibiotic treatment. He had spent some time in Muskoka earlier this summer and had been swimming in the lakes and rivers.

Eosinophilic bronchiolitis was identified on a bronchoalveolar lavage with several large trematode ova within the sample (Figure 1). The ova were typical of *Paragonimus kellicotti*. The adults of this trematode develop in cysts in the lung of both dogs and cats; animals infected with *P. kellicotti* may be asymptomatic or be presented with a variety of respiratory signs, including coughing, dyspnea, pneumothorax, bronchiectasis, and hemoptysis.

Both dogs and cats are susceptible to infection with *P. kellicotti* following ingestion of crayfish or paratenic

hosts infected with metacercariae. The prepatent period is 3 to 5 weeks or longer, and that is consistent with the dog's visit to Muskoka.

There are no products labeled for treatment of trematodes in dogs and cats; however, praziquantel, epsiprantel, and fenbendazole have been reported to be effective. Trematode eggs are less buoyant than those of nematodes or protozoa, unless high-density sucrose flotation is used. **Diagnosis of infection by fecal examination requires concentrating the ova present in feces by sedimentation rather than flotation.** *AHL*

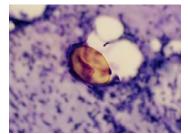


Figure 1. Ovum of *Paragonimus kellicotti* in the bronchoalveolar lavage from a dog.

Important notice - As per the AHL User's Guide and our submission forms: "The submitter acknowledges and agrees that the AHL may share test results and contact information as it deems necessary for the purposes of relevant legislation regarding reportable or notifiable diseases and for the purpose of surveillance of animal or public health in Ontario." **Please include owner contact information so that CFIA can followup and support international trade.** *Thank you!* URL for reportable/immediately notifiable diseases <u>http://www.inspection.gc.ca/english/anima/disemala/guidee.shtml</u>

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

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