UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

Volume 7, Number 3, page 25 September, 2003 ISSN 1481-7179

In this issue:

We're ISO 9001:2000 registered!

The Laboratory Services Division was audited to the ISO 9001:2000 standard by BSI Management Systems in February 2003 and is now compliant with this standard. We have been ISO 9002:1994 registered for several years, but this standard has been superseded by 9001:2000, with its increased emphasis on process management and client satisfaction. We are very pleased that our staff, with the support of the Lab Services' Quality Assurance Unit, have pulled together to ensure that we have in place a superior Quality Management System.

Congratulations Dr. Martin!

We are delighted to announce that **Dr. Emily Martin, AHL Pathologist, Avian and Fur-Bearing Species,** passed the American College of Poultry Veterinarians (ACPV) examination in July 2003, and is now a "**Diplomate ACPV**". Only 2 Canadians wrote the exam, and they were among the 5 that passed the exam. Emily joined the AHL in September 2000. A 1997 graduate from the Ontario Veterinary College, University of Guelph, she subsequently completed an MSc (2000) in Avian Pathology from the same institution.

Dr. Martin provides pathology evaluation for a diverse range of species including primarily avian species (poultry, game birds, pigeons, pet birds) as well as fur-bearing production species and exotics.

Purolator account number

Please use the current University of Guelph Purolator account number **0966901** on all 'incoming collect' specimen shipments to the AHL from within Ontario.

We would be happy to order **preprinted waybills** for you. Please do not order them directly from Purolator. For more information on completing waybills, please see page 5 of the Fee Schedule, or our handout "Packaging and shipping lab submissions".

Proficiency panel success!

The AHL routinely participates in a number of proficiency testing programs. **Congratulations to Maria Osuch** who successfully passed the International Leptospirosis MAT Proficiency Testing Scheme, a collaborative project of the Royal Tropical Institute, Amsterdam, The Netherlands; the Public Health Laboratory Service, Hereford, United Kingdom; and the National Serology Reference Laboratory, Melbourne Australia. This proficiency testing scheme was designed to provide quality assurance to laboratories performing the **microscopic agglutination test (MAT)** for either human or veterinary diagnosis of leptospirosis. A total of 59 laboratories from 34 countries participated: only 37 of 59 laboratories (63%) correctly identified the serogroups in all positive samples.

Improving communications with clients

Grant Maxie *gmaxie@lsd.uoguelph.ca*

Lab Services sends out a **'Service Check' form** with each of our monthly billing statements. Since we allow billing of owners, these requests are also sent to owners.

As well as compliments on our service, we receive occasional complaints from owners:

"Results not in lay terms".
"Very slow reporting time."
"Animal is either better or dead by the time the lab report is back."
"I never received the results. I have no idea of what the results were."
"Need to give more specific information re treatments."

We have made repeated attempts to inform owners of the role of the lab in order to prevent misunderstandings:

- We print a standard note on the bottom of every AHL report "For interpretation of results, please contact your veterinarian".
- An "Information for Owners" handout is given to owners who are dropping off submissions in person at our Guelph or Kemptville labs, and is posted on our website.
- Also posted on our website is this note: "Animal owners please note: This website is for a veterinary diagnostic laboratory. The AHL does not provide veterinary medical advice, consultations or referrals over the Internet. Please contact your veterinarian about such matters."
- We re-iterated in the March 2002 AHL Newsletter that we depend on practicing veterinarians for rapid communication of results to owners and for local follow-up action.
- We fax or email reports to producers in order that they are aware that we have reported test results to their veterinarian.

Are other solutions available?

We recently revised our standard note on AHL reports to be more comprehensive, and placed it immediately below our reports record:

"Note to owners: The AHL provides specialized diagnostic services to support your veterinarian, and has reported to your veterinarian as noted in the 'REPORTS' record above. For interpretation of this report, please contact your veterinarian. The AHL does not give treatment or management recommendations; your own veterinarian is best placed to give such advice."

We sincerely appreciate all efforts extended by veterinarians to report and interpret lab findings to their clients as promptly as possible.

We welcome input from our clients on any other means that we might use to improve client satisfaction. Please feel free to contact us. *AHL*

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AHL Lab Reports

CATTLE

Johne's disease testing: we're ready!

Marie Archambault, Jason Eidt, Hugh Cai, Davor Ojkic, Grant Maxie, Jocelyn Jansen, Steve Hendrick, Todd Duffield, Ken Leslie, Dave Kelton, Kerry Lissemore *marchamb@lsd.uoguelph.ca*

Johne's disease, also known as paratuberculosis, is an infectious disease of ruminants caused by *Mycobacterium avium* subsp *paratuberculosis* (MAP). This organism causes chronic granulomatous enteritis and is considered a serious infectious disease affecting the world's cattle industry.

We designed a collaborative project to provide rapid and accurate 'in-house' diagnostic tests to identify cattle infected with MAP in Ontario. The main objective was to validate the BACTEC fecal culture system and the IDEXX PCR DNA test kit to identify MAP in bovine feces. The BACTEC 460TB liquid culturing system is a radiometric detection method that can detect MAP in lower numbers and faster than can traditional culture. It can also grow MAP from a variety of species, including sheep. The analytical validation is now done and the field validation is almost complete! We are now ready to share results and recommendations on how to use these tests.

Fecal, serum and milk samples were collected from 33 dairy herds in southwestern Ontario with a suspected high prevalence of Johne's disease. Serum samples were tested at our laboratory for antibodies with the Johne's HerdChek IDEXX ELISA. Milk samples were tested by Antel BioSystems Inc. (Lansing, MI, USA) with an in-house milk ELISA. Fecal samples from 326 cows identified as positive on either the serum or milk ELISA were tested by traditional fecal culture (AntelBio), IDEXX fecal PCR (AHL), and BACTEC culture (AHL).

Of the 326 fecal samples tested, 89 (27%) were positive on direct PCR, 144 (44%) on traditional fecal culture, and 186 (57%) on BACTEC culture (Figure 1). **Although traditional fecal culture is recognized as the gold standard, BACTEC culture appears more sensitive than traditional fecal culture, based on our results to date.** This observation has been reported previously (1,2).

Statistical results were calculated for 257 cows having milk and serum ELISA's, fecal PCR, BACTEC culture, and traditional fecal culture results. The positive predictive value (PPV) for the milk ELISA compared to traditional fecal culture was 61%. The PPV for the serum ELISA compared to traditional fecal culture was 45%. The kappa statistic between fecal PCR and traditional culture was 0.57. A kappa of 0.66 was calculated for BACTEC culture versus traditional culture. Previous research has shown that organism-based tests are more sensitive and specific (3). Our data also show that the milk ELISA (61% sensitivity, 95% specificity) performance is comparable to the serum ELISA (76% sensitivity, 88% specificity). The sensitivities of ELISA's in this study are higher than previously reported, likely due to the bias in herd selection based on higher prevalence.

To evaluate BACTEC culture turnaround time, numbers of Johne's-positive fecal samples by week were evaluated for 84 samples; 48 of them were found positive (Figure 2) and the majority of positive samples (38) were detected within 4 weeks. Traditional culture can take up to 16 weeks to complete. The turnaround time for the direct fecal PCR is 3 to 5 working days, but this test appears to be less sensitive than traditional and BACTEC cultures.

(continued on page 28)

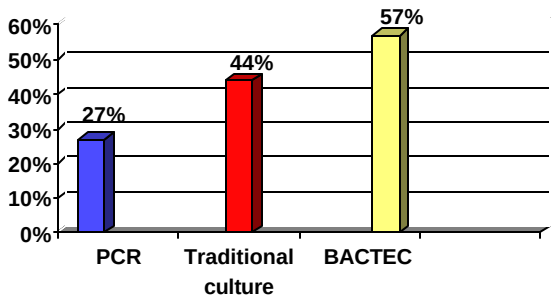

Figure 1. Positive fecal test results for MAP from 326 ELISA-positive cows.

Johne's disease testing - *continued from page 27*

The United States Animal Health Association (USAHA) Scientific Advisory Subcommittee on Johne's disease has provided recommendations on the use of serology tests for detection of the disease (4). The following recommendations were made and may be useful to practitioners:

- The ELISA should be the primary screening tool, followed by an organism-based detection test.
- Low prevalence herds should monitor their status with an organism-based detection test (more sensitive).
- If the lab reports a high number of ELISA-positive cows, validation of the assay should be confirmed by 'in-house' controls and based on manufacturer's information, and the lab should recommend that the test on those samples be repeated. If samples are still positive, an organism-based detection test should be used to confirm those ELISA-positive cows in the herd, or a valid subset of cows should be tested.

In December 2002, at the Canadian Animal Health Consultative Committee, producer organizations and provinces accepted in principle a control program to reduce the prevalence of Johne's disease in Canada. Likely to commence late 2003 or in 2004, the program will be voluntary. Provincial departments of agriculture will likely be responsible for programs, and implementation may vary from province to province (5-8). Alberta already has a voluntary Johne's control program (9). Meanwhile, Ontario practitioners can voluntarily send samples to the AHL for testing. We're ready!

Current AHL fee information

Serum ELISA is $7.50/sample, BACTEC culture is $25/fecal sample, and direct PCR is $20/fecal sample.

Acknowledgments

The Food Safety Research Program, Innovation and Risk Management Branch of OMAF; Dairy Farmers of Ontario; Elanco Animal Health; Antel BioSystems Inc.; and Ontario DHI supported this research. We also acknowledge the 33 Ontario dairy producers, Animal Health Monitoring Lab of British Columbia, technicians and summer students for their assistance. *AHL*

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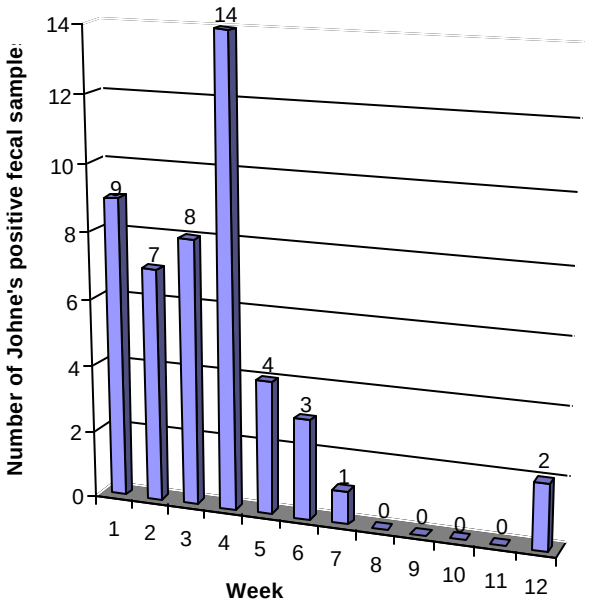

Figure 2. Number of MAP-positive fecal samples by week, using BACTEC culture on a subset of fecal samples.

Outbreak of clinical salmonellosis in two Ontario dairy herds caused by a multidrug-resistant (MDR) *Salmonella* Newport isolate

Marie Archambault, Ann Godkin *marchamb@lsd.uoguelph.ca*

Recently, two Ontario dairy herds have experienced an outbreak of clinical salmonellosis due to a multidrug-resistant (MDR) *Salmonella enterica* serovar Newport isolate.

- On both farms, mature cows developed **watery to bloody diarrhea**, fever, were off feed, dehydrated, and had a sudden drop in milk production.
- **High morbidity** with some mortality was observed.
- Clinical disease occurred predominantly in young **periparturient cows** and **very young calves**.
- These cases were **unresponsive to antibiotics.**

The first positive isolate came from a cow in herd A cultured March 17, 2003 at the AHL. The isolate was identified as an MDR *Salmonella* spp. Health Canada subsequently identified and confirmed the MDR *Salmonella* serotype Newport. The second positive isolate came from neighboring farm B on June 17, 2003.

A veterinarian from OMAF Veterinary Science and the private practitioner visited both farms. During the farm visits, fecal swabs were collected from all cattle on both farms and submitted to AHL. These were cultured in pools of 3. Samples from individual animals contributing to a positive pool were then cultured. If the pool was negative, the individual samples were not cultured.

While MDR *Salmonella* Newport has only been isolated from 3 cattle herds in Ontario to date, this agent does exist as a potential threat

Farm A

At the time of the first positive culture from a sick cow on farm A, 12 animals were reported as sick, 4 were dead and 177 were deemed at risk. One month later, at the time of the investigative visit, 3 more cows had died or had been euthanized. It was estimated that about 15 lactating animals had diarrhea at that time. Some of these were off feed and dehydrated. Two previously ill cows had been treated and recovered. About 6 weeks prior to the first sick cow in March, the owner reported an outbreak of calf diarrhea in 10-day-old calves; 5 had died. A calf submitted for post-mortem at that time was negative for *Salmonella* spp and positive for cryptosporidiosis.

Subsequent to the calf problem, a higher level of hygiene had been adopted, and no calves had become sick since. One farm A employee had recently had diarrhea, which had been confirmed as salmonellosis. Twenty-nine isolates of MDR *Salmonella* spp were recovered from 173 animals tested on this farm. All isolates were forwarded to Health Canada for serotyping. All of them were MDR *Salmonella* Newport. Follow-up cultures were performed approximately 2 months after this investigation to re-evaluate the status of the herd; 184 samples were submitted and 15 MDR *Salmonella* spp isolates were recovered. Serotyping is pending on a subset of those. **Infection was present in all age and housing groups on farm A**. The high morbidity over time without clinical disease suggested that infection was widespread but that clinical disease was relatively rare. Most animals had cleared infection by the time of the second culture. Two calves were positive on both cultures.

Farm B

Farm B is adjacent to farm A. Approximately 75 animals were at risk. Six cows had scours with high fevers (106-107F) with no deaths at the time of the first submission in early June. Family members (including a 5-month-old baby) and two employees also had pyrexia and diarrhea during the next month. MDR *S.* Newport was identified from the first 4 fecal swabs submitted from the initial clinical cases. During the investigation, 75 fecal samples were collected on July 4. These included fecal samples from dry cows, bull, heifers, milking cows, and calves. Thirty-two bovine milk samples, 4 cat fecal swabs and 2 horse fecal swabs were also submitted. There were 31 isolates of MDR *Salmonella* spp from 82 animals. The milk samples and the 2 horses were negative, but 3 cats were positive. Serotyping is in progress. **Among cattle, positive test results were clustered among the youngest calves**. Older heifers, housed in close proximity to the sick pen where scouring cows had been held 1 month previously, also were positive.

These case reports suggest that **MDR *S.* Newport may be a differential diagnosis for outbreaks of diarrhea on dairy farms where dysentery in any age of animal and pyrexia are occurring.** While MDR *Salmonella* Newport has only been isolated from 3 cattle herds in Ontario to date, this agent does exist as a potential threat. The resistant nature of this serotype may have implications for antimicrobial therapy on affected farms. **The widespread dissemination of infection found following the outbreak of clinical disease was also noteworthy.**

(continued on page 30)

MDR *Salmonella* Newport - *continued from p 29*

If you suspect infection with MDR *S*. Newport, fecal samples can be submitted to the AHL for bacterial culture and antimicrobial susceptibility testing. Antimicrobial use should be limited to dire cases only. A **strict biosecurity program**, targeted towards reducing manure contamination between groups of cattle within and between farms, should be initiated. Clinically affected cows should be treated with fluids, either oral or IV, and appropriate non-steroidal anti-inflammatories. Encourage clients **to contact their physician** if concerned about concurrent human illness.

More information on *Salmonella* spp in cattle populations can be found at the National Animal Health Monitoring System (NAHMS) website: http://nahms.aphis.usda.gov Data on control points and biosecurity for *Salmonella* spp infections in cattle can be found on the NYSCHAP website at: http://nyschap.vet.cornell.edu/ *AHL*

To protect the health of people on affected farms:

- **Warn clients of the dangers of drinking raw milk.**
- **Implement scrupulous handwashing.**
- **Store barn clothes and boots away from the house.**
- **Keep children away from the barn and manure until they are old enough to understand and carry out the recommended hygienic measures.**

Ontario BSE surveillance update

Murray Hazlett, Josepha DeLay, Paul Innes *mhazlett@lsd.uoguelph.ca*

BSE testing at the AHL has approximately doubled since the occurrence of the single positive case in Alberta this spring. We have had a number of enquiries about our testing, and we hope to answer many of the questions here. Although this is a federally reportable disease, funding of the AHL's BSE laboratory is through the Ontario Ministry of Agriculture and Food, with technical support by the reference CFIA laboratory in Winnipeg. We are a member of the National TSE Veterinary Diagnostic Laboratory Network.

Since starting immunohistochemistry testing for BSE in March 2002, the AHL has tested 1300 target animals (mostly downer or deadstock cattle). Specimens are collected and submitted by specially trained provincial livestock inspectors. Samples from held carcasses at provincially inspected abattoirs usually have test results done within 48 hours of receipt of the specimen, while those from condemned carcasses or deadstock have a 3 to 7 day turnaround.

More emphasis has been given recently to the sampling of deadstock animals, and these now make up about 50% of samples. To correct a misconception, not all downer animals or deadstock are tested. Most are not tested at this time. **Currently, we test a random sample of mature (>24 months) animals - about 50% of samples are from downer animals, and 50% from deadstock**. *AHL*

IBRV abortions, winter 2003

Peter Lusis, Beverly McEwen, Susy Carman, Katie Welch *plusis@lsd.uoguelph.ca*

From January to March 2003, 26 abortions due to infectious bovine rhinotracheitis virus (IBRV) were received from 6 herds. Compared to 1999-2002, this is an increase over the average of 3 herds/year (range 0-5) with 6 abortions/herd (range 0-14). Because of submission biases to the AHL, these data may not reflect a true increase in abortion due to IBRV in the Ontario cattle herd. However, **submission of fetuses and placentas is recommended in cases of abortion to maintain provincial surveillance and herd-level diagnosis**.

IBRV abortions typically occur a few weeks after maternal infection, and are usually not associated with clinical signs of respiratory or other diseases in aborting dams or in the herd. In several herds, abortions were multiple (over a few days or weeks), and some abortions followed introduction of new cattle into a herd, either in the resident or newly-introduced cattle. Histories were often incomplete, and we therefore cannot assess the significance of various risk factors, such as vaccination.

Virus isolation and histopathology are the methods of choice for IBRV abortion diagnosis. Acute and convalescent serology is also useful in diagnosis of IBRV infection. *AHL*

POULTRY

Salmonella enterica serovar Arizonae in turkey poults

Brian Binnington, Marie Archambault, Keith Harron *bbinning@lsd.uoguelph.ca*

Increased mortalities occurred from day 1 in a flock of 14,000 turkey poults. The poults had been given a neomycin-chlortetracycline product on day 9, and poults were submitted to the AHL on day 10. The live birds were depressed and a few had fine head tremors.

On gross necropsy, the stomachs of all poults contained litter, and approximately half of the birds had an empty crop. A few of the birds had watery intestinal contents, but in the majority of the poults the contents appeared normal. A few poults had a hard yolk sac remnant adhering to the intestine and umbilicus.

Microscopic examination of brain and spinal cord demonstrated intense fibrinoheterophilic inflammatory exudates in meninges and ventricles. Numerous rod-shaped bacteria were present in the exudates (Figure 1). Multiple thrombosed blood vessels were present in the meninges and the parenchyma of the brain.

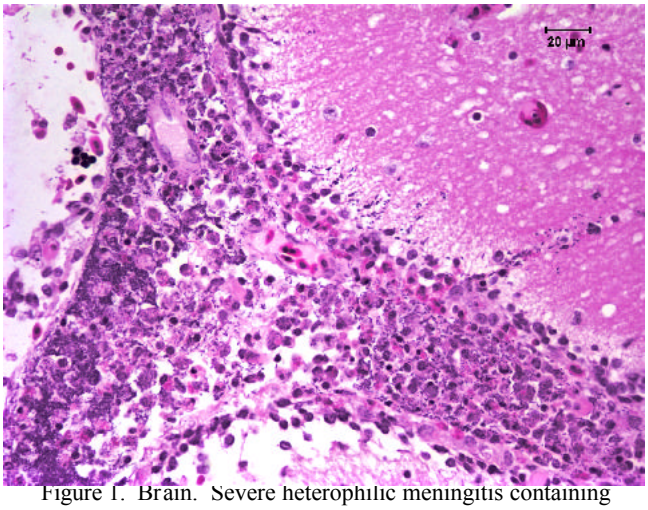

Figure 1. Brain. Severe heterophilic meningitis containing numerous bacteria.

Large numbers of heterophils were present in the thrombosed vessels and in the surrounding parenchyma. Yolk sacculitis with fibrous membrane thickening, accumulations of heterophils, mononuclear cells and bacteria was present in several of the poults.

Large numbers of *Salmonella enterica* serovar Arizonae, subspecies IIIa:18:-:-, were isolated from livers and yolk sacs but not from cecal contents. This organism was susceptible to most of the antibiotics tested including neomycin, but it was resistant to tetracycline.

Large numbers of *Salmonella enterica* serovar Arizonae, subspecies IIIa:18:-:-, were isolated from livers and yolk sacs

The diagnosis was bacterial meningoencephalomyelitis and septicemia compatible with arizonosis.

Salmonella Arizonae can cause significant disease in young poults and chicks. Mortality rates of 10 to 50% have been reported in the first week after hatching, and mortalities can continue for 2 to 3 weeks. Infection of the eyes is a frequent finding, and recovering birds can have severe persistent infection of the eyes. Intestinal carriage and shedding of the bacteria can persist in adult birds for a prolonged period of time. Infection of the ovary and transmission via the egg can occur.

In this case, it was reported that the poults were sourced from the USA. Regular screening of breeder turkeys and chickens is conducted through the Ontario Hatchery and Supply Flock Policy (OHSFP). Approximately 10,000 environmental and fluff samples are cultured annually, and samples from turkey breeders comprise approximately 2,000 of these samples. OHSFP records from the year 2000 to the present have no isolates of *Salmonella* Arizonae from Ontario breeders during this period. *AHL*

Reference

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Encephalitis in 7-week-old turkey poults

Brian Binnington *bbinning@lsd.uoguelph.ca*

During the winter of 2003, the AHL received samples of brain, lung and serum from a flock of 16,000 turkeys that had a **clinical history of lameness, torticollis and pneumonia in 7-week-old poults**. Histological examination of the lung samples demonstrated multifocal granulomatous inflammation containing septate fungal hyphae. The pieces of submitted brain had prominent accumulations of mononuclear cells (lymphocytes, macrophages) and a few heterophils in the meninges and in the perivascular space surrounding parenchymal blood vessels. No structures suggestive of fungi could be identified in the sections examined (Figure 1). *Aspergillus fumigatus* was isolated from lung samples, but not from submitted brain swabs. Serological testing of 9 serum samples was not indicative of avian influenza or Newcastle disease.

Aspergillus fumigatus is a significant pathogen in turkey production

A second sample of live and dead birds was submitted to investigate the on-going problem and rule-out the possibility of a viral infection. Approximately 500 birds were sick and 150 had already died. The sick birds were "going off their legs" with twisted necks in some birds or paddling of legs in other birds. On gross necropsy, the poults had miliary yellow nodules throughout the lungs, and yellow, firm nodules or plaques in the air sacs. One poult had a 0.5 cm diameter yellow caseous lesion in the cerebrum and three others had 3 to 4 mm diameter nodules in the cerebellum and/or brainstem. Histological examination demonstrated multiple granulomatous foci in the lungs and air sacs that contained numerous septate fungal hyphae. In sections of brain, there were areas of necrosis with neuropil malacia, vasculitis and thrombosis that corresponded with the lesions observed on gross examination. Numerous branching, septate fungal hyphae were present in the thrombosed blood vessels and in the areas of necrotic neuropil (Figure 2). Perivascular lesions similar to those observed in the first submission were present in brain adjacent to the necrotic areas and around the ventricles. Prominent cuffs of mononuclear cells without vasculitis, thrombosis or the presence of fungi characterized these perivascular lesions.

Samples of brain, cecal tonsil and trachea were submitted for virus isolation. No virus was detected after 3 passages in SPF embryonated chicken eggs.

A diagnosis of mycotic pneumonia, airsacculitis and encephalitis due to *Aspergillus fumigatus* was made.

Aspergillus fumigatus is a significant pathogen in turkey production. Disease is most frequently associated with the respiratory system, but fungemia and disseminated lesions to other tissues such as brain can occur, especially in young birds. In this case, the brain lesions (perivascular mononuclear cell cuffing) seen in the first submission were not typical of a fungal lesion, and agents such as a virus had to be considered. On the follow-up submission of affected birds, typical lesions of mycotic encephalitis were evident when whole brains were examined. It is likely that the limited sampling of brain on the first submission had failed to identify the fungal cause of the encephalitis. *AHL*

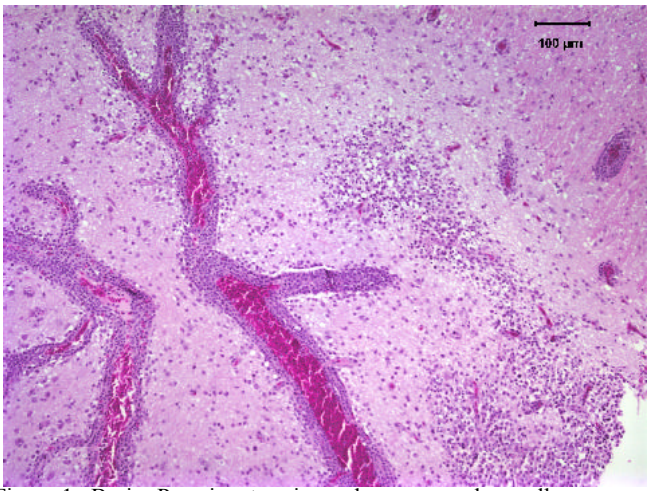

Figure 1. Brain: Prominent perivascular mononuclear cell accumulation seen in poults of first submission

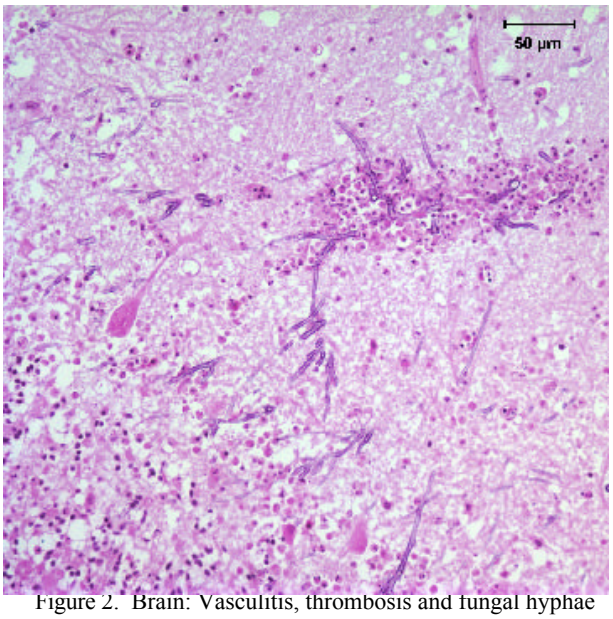

Figure 2. Brain: Vasculitis, thrombosis and fungal hyphae seen in poults of second submission

SWINE

A case of tiamulin and narasin toxicity in nursery pigs

Gaylan Josephson, George Charbonneau, Brent Hoff *gjosephs@lsd.uoguelph.ca*

Piglets from a 500-sow farrow-to-finish operation were weaned at approximately 18 days of age, into a nursery that was operated as all-in/all-out by room. On June 6, 2003, 7 days post-weaning, approximately 3% of the pigs showed signs of lack of appetite and appeared in fact, to be losing weight. The number of pigs affected and severity of clinical signs increased, and within 4 days, over 15% of the piglets were affected. **Pigs were showing increased signs of weakness, ataxia and incoordination, to the point of recumbency.** Paddling was not a feature, nor were other neurological signs observed.

Differential diagnoses included selenium toxicity and an unusual manifestation of *Streptococcus suis* septicemia, although clinical signs were not fully supportive of these diagnoses. **Since tiamulin was included in the ration, ionophore toxicity had to be considered**. Considering the possibility that this might be a *Streptococcus suis* infection, the pigs were treated with a water-soluble form of penicillin. The citric acid that was being used for post-weaning scour control was eliminated from the water supply, in case it might be contributing to the problem. Beneficial effects were not identified. Investigations failed to reveal either a water or a stray voltage problem.

The toxic effects of narasin and tiamulin are synergistic

At this time, it became apparent that only pigs consuming the phase 2 ration were affected. This ration contained 220 g chlortetracycline and 31.2 g tiamulin (Denagard) per tonne of feed. The feed bin had been emptied prior to this group of pigs arriving in the nursery, so they were the first to receive feed from the newly filled bin. Feed was delivered to the pigs in a dedicated feed cart. The first observable clinical signs were noted 4 days after starting this feed. The suspect feed was immediately removed and replaced.

Five days after the appearance of clinical signs, 166 of 433 pigs were obviously losing condition, many were weak and "wobbly" on their feet, with 4 unable to stand. At this time, several animals weighing between 4.3 and 5.7 kg were submitted to the Animal Health Laboratory.

Significant lesions were not noted on the carcasses of any of the submitted piglets. Microscopic lesions were confined to skeletal muscles – including several sections from each animal, with all being similarly affected. **Myodegeneration was marked, involving up to 100% of myofibers in some sections of muscle.** In the most severely affected areas, cross-striations were not visible, and some fibers appeared amorphous, hypereosinophilic and swollen. However, most fibers were undergoing regenerative repair, with invasion by macrophages. Mineralization of muscle fibers was not apparent.

The feed supplier checked the batch sheets, and nothing abnormal was noted. Further investigation identified a mechanical problem with an ingredient auger that might have allowed the inadvertent introduction of narasin (an ionophore) into the phase 2 ration. **Testing of feed revealed levels of narasin in the suspect feed to be in excess of 81 mg/kg (ppm).** This, along with the presence of tiamulin in the feed, would be consistent with the clinical signs observed.

Narasin (Monteban) is an ionophore used primarily in chicken feed as a coccidiostat or to prevent necrotic enteritis. It is licensed for use in swine as a growth promotant, at the level of 15 g per tonne of finished feed, but very little is used in Ontario. **Product labels clearly state that narasin is not to be fed if tiamulin is being used**. The toxic effects of narasin and tiamulin are synergistic, and are believed to be due the inhibition of ionophore biotransformation by microsomal enzymes in the liver. Evidence suggests that the damage is ultimately due to calcium overloading.

By day 15, approximately one-half of the 430 pigs were markedly unthrifty, 25% were less severely affected, and only 25% could be considered as "normal".

Deleterious long-term effects of tiamulin/narasin toxicity have not been reported in swine. Growth was markedly reduced in this group of pigs, and they were not performing well 2 weeks after the contaminated feed had been removed.

Given the difficulty in ascertaining the proper withdrawal time for the combination of narasin and tiamulin, **it was recommended that all 430 pigs be euthanized and buried on site**, in order to avoid the risk of their introduction to either the human food chain or the rendering system. *AHL*

Discontinuation of the Sarcoptest ELISA

The manufacturer is discontinuing production of the Sarcoptes ELISA. We have only a small supply of strips left, so please call Dr. Davor Ojkic (519-824-4120, ext 54524) before sampling pigs.

HORSES

Equine abortion diagnoses, 2002/2003

Beverly McEwen, Marie Archambault, Susy Carman, Murray Hazlett *bmcewen@lsd.uoguelph.ca*

Equine abortion diagnoses have not changed substantially over the past 4 years, although the frequency of abortion submissions decreased slightly this past year (Table 1). The submissions in 2002/2003 were received from 61 owners, however, owner identification was not given in 31 cases, making diagnoses at the herd-level problematic. Twelve breeds were represented. Thoroughbred abortion submissions were over-represented at 36% compared to the overall AHL case submission rate of 26%, whereas Standardbred abortion submissions of 24% were lower than the overall AHL submission rate for Standardbreds of 30%.

Infectious abortions collectively comprised 37% of all abortion diagnoses, followed by non-infectious causes of abortion, primarily umbilical torsion (12%). Of infectious causes, **equine herpesvirus type 1 (EHV-1) remains the most frequent single abortifacient pathogen identified, accounting for 12% of all abortion diagnoses**. However, 6 of the EHV-1 abortions were submitted from 2 premises with abortion storms. *AHL*

Table 1. Equine abortion diagnoses, AHL fiscal years, 1998/1999 - 2002/2003

Fiscal year	**98/99**	**99/00**	**00/01**	**01/02**	**02/03**
Number of abortion cases submitted	64	89	89	97	92
Frequency of abortion of total AHL equine submissions	1.3%	1.7%	1.5%	1.5%	1.1%
Pathology diagnoses (number, %)*					
Equine herpesvirus-1 (EHV-1)	**9**	**8**	**13**	**10**	**11**
Proportion of equine abortions due to EHV-1	14%	9%	15%	10%	12%
Non-viral infectious abortion, combined	**17**	**25**	**16**	**21**	**23**
Proportion non-viral infectious abortion	27%	28%	18%	22%	25%
Streptococcus zooepidemicus	1	5	1	4	6
Staphylococcus aureus	0	3	0	0	0
Streptococcus equisimilis	2	0	0	1	2
Ehrlichia risticii	0	0	0	0	0
Klebsiella sp	0	0	1	0	0
Leptospira sp	3	0	0	1	1
Nocardia sp	0	0	1	0	0
Placentitis	8	7	6	11	13
Miscellaneous bacteria/fungi	2	8	0	0	0
Mycotic	1	0	1	2	0
Lesions compatible with bacterial	0	2	6	2	1
Non-infectious causes of abortion, combined	**15**	**21**	**24**	**26**	**18**
Proportion, non-infectious causes of abortion	23%	24%	27%	27%	20%
Umbilical torsion	8	12	8	15	11
Placental edema	4	3	8	0	1
Placental mineralization	1	0	0	4	3
Placental adenomatous/cystic hyperplasia	0	1	0	0	2
Fetal goiter	0	0	1	1	1
Dystocia/stillbirth	2	5	7	6	4
Congenital anomalies	0	0	0	0	0
Idiopathic abortions	**24**	**38**	**36**	**42**	**39**
Proportion, idiopathic abortions	38%	43%	40%	43%	42%

* Number of diagnoses may add to more than the number of cases submitted because more than one diagnosis was made in some cases.

Idiopathic polyradiculoneuritis in a horse

Sameh Youssef *syoussef@lsd.uoguelph.ca*

A 6-year-old Standardbred horse was euthanized and submitted for postmortem examination after a 1-week history of dysphagia, laryngeal hemiplegia, and hindlimb paralysis that progressed in the last 2 days to lateral recumbency and occasional paddling. Gross lesions were non-specific. Histologically, mild-to-moderate lymphocytic and plasmacytic polyradiculoneuritis with mild axonal swelling and demyelination were present particularly in the ventral (motor) spinal nerve roots of the cervical and lumbar spinal cord. In addition, mild lymphocytic and plasmacytic ganglioneuritis was observed in the associated spinal ganglia. Extraneural lesions were limited to mild pulmonary edema.

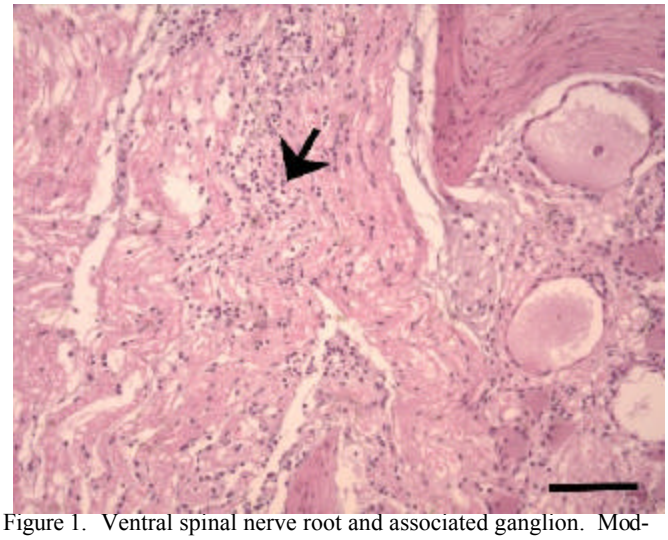

Figure 1. Ventral spinal nerve root and associated ganglion. Moderate, focally extensive, mononuclear cell infiltrate. Bar = 500 µm.

A diagnosis of idiopathic polyradiculoneuritis and ganglioneuritis (IP) was made after exclusion of common infectious and non-infectious causes of equine polyradiculoneuritis, which include infection with the paralytic strains of equine herpesvirus (EHV 1/4), *Neospora* spp., infestation with *Halicephalobus gingivalis*, and cauda equina neuritis. Rabies was also excluded by using viral culture and FA testing. IP is uncommon in dogs and cats, and extremely rare in horses. A presumptive immune-mediated pathogenesis was suggested for canine IP, however the pathogenesis in the few reported equine cases is unclear.

Equine IP is usually associated with cauda equina neuritis. In this case, the cauda equina was normal, and the polyradiculoneuritis was lymphoplasmacytic rather than granulomatous, as is expected in most of the cases associated with cauda equina neuritis. In conclusion, **histologic examination of spinal nerve roots should be included in the routine examination of equine neurologic cases particularly when paralysis and/or recumbency are major clinical signs**. *AHL*

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1. Summers BA, Cummings JF, De Lahunta A. Veterinary Neuropathology. Mosby-Yearbook, St. Louis, Missouri. 1995.
2. Daft BM, Barr BC, Collins N, Sverlow K. *Neospora* encephalomyelitis and polyradiculoneuritis in an aged mare with Cushing's disease. Equine Vet J 1997; 29: 240-243.
3. Fordyce PS, Edington N, Bridges GC, Wright JA, Edwards GB. Use of an ELISA in the differential diagnosis of cauda equina neuritis and other equine neuropathies. Equine Vet J 1987; 19: 55-59.

Tests introduced by the AHL in 2002/2003

Immunohistochemistry

- bovine spongiform encephalopathy (BSE)
- chronic wasting disease (CWD)
- bovine viral diarrhea virus (BVDV)
- bovine coronavirus (BCV)
- infectious bovine rhinotracheitis virus (IBRV, BHV-1)
- porcine circovirus-2
- *Toxoplasma gondii*
- *Leptospira* spp.
- West Nile virus
- cell markers

  actin, CD3, CD79a, CD18, chromogranin, cytokeratin HMW & AE1/AE3, factor VIII-related ag, GFAP, melan-A, neuron-specific enolase, S100, thyroglobulin, synaptophysin, vimentin

Polymerase chain reaction (PCR)

- *Mycoplasma hyopneumoniae*, lung tissue
- PRRS virus sequence analysis
- *Salmonella enterica* serotype Typhimurium DT104
- 16S RNA gene sequence analysis for bacterial ID
- swine influenza virus PCR/typing
- West Nile virus RT-PCR
- scrapie resistance PrP genotyping
- *Neorickettsia (Ehrlichia) risticii*
- infectious bursal disease virus typing PCR
- infectious bronchitis virus phylogenetic analysis

Other

- West Nile virus IgM ELISA, equine
- West Nile virus IgG ELISA, equine
- Johne's disease test validation – BacTec culture, IDEXX PCR
- ICP mineral deficiency screens

COMPANION ANIMALS

Infectious canine hepatitis in a puppy

Beverly McEwen, Josepha DeLay, Susy Carman *bmcewen@lsd.uoguelph.ca*

A mixed-breed, stray, 6-week-old female puppy, under veterinary care for malnutrition, began to seizure uncontrollably and died. At post-mortem, the puppy was emaciated and dehydrated. In liver, there were both small focal areas of coagulation necrosis of hepatic parenchyma and single cell necrosis of hepatocytes. Large amphophilic intranuclear inclusions in hepatocytes, Kupffer cells, and sinusoidal endothelium stained positively with antibody to adenovirus by immunohistochemical methods on formalin-fixed tissue. In brain, low to moderate numbers of neutrophils, macrophages, and lymphocytes were present in meninges and intermingled with fibrin and hemorrhage in Virchow-Robin spaces surrounding cerebral and cerebellar blood vessels, which were lined by reactive endothelium, occasionally containing similar adenoviral inclusions. Adenoviral inclusions were also present in renal glomerular capillaries. *Klebsiella oxytoca* was isolated from lung and spleen.

Canine adenovirus-1 infects a broad range of canids as well as bears. The virus initially infects tonsils and spreads to local lymph nodes, eventually resulting in viremia. **The virus has a predilection for hepatocytes, endothelial cells, and mesothelium, producing hepatic periacinar to panlobular and single cell necrosis as well as multiorgan hemorrhage**. As a result of current canine vaccination protocols, infectious canine hepatitis is now uncommon, with the last case recalled at this laboratory occurring in the mid-1980's.

Lesions of CAV-1 infection commonly involve liver, serosal surfaces, lung, kidney, lymph nodes, brain, and bone. Infected animals may be found dead, often as a result of cerebral hemorrhage, or may develop a range of clinical signs including vomiting, melena, pyrexia, and non-specific neurologic signs. Ocular involvement in the early stages of disease results in corneal edema due to infection of corneal endothelium; in later stages, following the production of neutralizing antibodies, uveitis can result from local immune-complex deposition and complement activation.

In this pup, either *K. oxytoca* septicemia or adenoviral infection alone could have resulted in death. *AHL*

References

1. Greene CE. Infectious canine hepatitis and canine acidophil cell hepatitis. In: Infectious Diseases of the Dog and Cat, ed Greene CE, 2nd edition, pp. 22-27. W.B. Saunders Company, Philadelphia, PA, USA, 1998.
2. Kelly WR: The liver and biliary system. In: Pathology of Domestic Animals, eds. Jubb KV, Kennedy PC, Palmer N, 4th ed., vol 2, pp 364-366. Academic Press, San Diego, CA, USA, 1993.

Client communications feedback

Inserted in this issue of the AHL Newsletter is a brief feedback form that we encourage you to complete and return to us. We produce the broad range of communications listed below to serve you better, and we look forward to receiving your feedback.

AHL Newsletter (March, June, September, December)
AHL Fee Schedule, May 1, 2003
AHL User's Guide
AHL LabNotes (BVDV testing, field PM's, bleeding birds, metabolic profiling)
Reporting of AHL lab results
AHL website http://ahl.uoguelph.ca