UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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Client Service Notes

Did you know?

- We require **owner postal codes** on **ALL** food animal submission forms in order to provide you with the OMAF financially supported food animal rates, and to allow us to meet our epidemiological information goals for OMAF.
- **Samples sent to other laboratories** for tests currently not available at the AHL have a turnaround time that starts once the samples arrive at that laboratory. The fees for these tests are "subject to change without notice", and therefore we don't list their prices in our Fee Schedule. We ship to those labs Monday through Thursday, depending on our internal workload; we guarantee Monday and Thursday shipping.
- We will **email** your reports to you if you provide your email address at submission.
- You need to protect the people who handle your sample shipments after they leave your clinic. Fetus and placenta submissions must be triple-bagged and tied off in heavy-duty garbage bags to prevent body fluids from seeping out of your parcel. These submissions should only be shipped in corrugated cardboard boxes to withstand the journey (see our User's Guide for more details). Paperwork should always be inside a separate plastic bag to keep it clean and dry. **Leaking samples and dirty paperwork can spread zoonotic disease.**

New AHL *Client Services Veterinarian* position

Dr. Gaylan Josephson retired on Dec. 31, 2003, from the AHL as our Swine Health Advisor/Food-animal Pathologist, after a total of 33 years of service to OMAF and the University of Guelph. We have restructured this position to include services to a wider range of practitioners, with emphasis on swine and cattle. Our New Client Services Veterinarian will facilitate the optimal selection and submission of specimens by practitioners, and provide expert consultative services for referring veterinarians about interpretation of test results and recommendations for further action. For more details, see the University job posting at **http://www.uoguelph.ca/HR/jobpost/040121b.htm .** The deadline for receipt of applications is March 15, 2004.

7 steps for optimal sample submissions & outcomes

Josepha DeLay and Linda McCaig

1. Call ahead if you need clarification on test selection and tissue sampling for your specific case. Choosing the best tests BEFORE sampling allows you to take appropriate samples that will produce useful test results. A pathologist or technologist is always available to answer your questions.

2. Be thorough and complete when filling out test requisition forms. Important information such as age and breed have significant implications for test methodology. For example, various culture media are selected to target organisms specific for different age groups of pigs.

(continued on page 2)

7 steps to optimal sample submissions and outcomes - *continued from page 1*

3. Provide a useful, concise history and differential diagnoses. Your clinical findings are the most significant part of the diagnostic process, as you are the one who actually sees the animal. Laboratorians must rely on your information and correlate this with results of laboratory tests in order to provide you with useful, clinically relevant information. Lab results taken out of context of the clinical situation can be misleading or, at best, not helpful.

4. Spin and separate serum samples prior to shipping. For CBC's, make 2 smears and submit the slides as well as the anticoagulated blood sample. Erythrocytes and leukocytes don't travel well, and smear preparation from fresh blood provides us superior cell preservation necessary for differential counts and assessment of cell morphology. In whole blood samples, leakage from blood cells will influence and alter biochemistry results.

5. Place samples for histopathology in formalin IMMEDIATELY upon collection. Autolysis begins as soon as an animal dies and results in progressive obliteration of useful morphologic detail. In gut, complete sloughing of the mucosal epithelium can occur in as little as 10 minutes. Because many important GI diseases (especially in neonatal animals) target epithelium, preservation of this tissue is vital to reaching a diagnosis. Open gut samples so that the mucosa fixes quickly. Samples of solid organs, e.g., liver, should be ≤1 cm thick for adequate fixation. To avoid freezing artifact in cold weather, fix tissues for 24 h prior to shipping.

6. Submit a SEPARATE tissue sample for each test that you request and place EACH tissue sample in a separate Whirl-Pak bag that is labeled with both the tissue and the animal / owner's name. Why bother separating tissues into individual bags?

- Prevents cross-contamination of tissues, which could lead to very confusing or erroneous test results!
- Ensures that the correct tissues are used for each test. Have you ever noticed that all fetal tissues tend to look alike? This is especially true when they are grouped together in one bag and must then be separated for individual tests!
- Expedites transfer of your samples to individual labs for testing. The extra time required for sample handling (splitting, labeling, separating) results in a considerable lag between sample receipt and testing.

7. Package your samples appropriately. This sounds simple but is often overlooked, and results of poor packaging can be devastating. *We all know that the samples that were the most difficult to obtain are the ones most likely to be damaged in shipping!* Use gel freezer packs, NOT ice cubes - they melt! Use plastic containers only (no glass) for formalin-fixed tissues, and double-wrap all containers and Whirl-Paks in leakproof plastic bags. Paperwork should be enclosed in separate plastic bags. Shock-absorbent packing such as styrofoam chips or crumpled newspaper will protect your samples, and be sure that the box or other container used is strong enough to survive the shipping experience. *AHL*

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Its mission is to inform AHL clients and partners about AHL current activities, and lab-based animal disease events and disease trends.

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Zoonotic pathogens and diseases identified at the AHL, 1999 - 2003

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Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens: in 2003, AHL staff identified **eastern equine encephalitis (EEE) , methicillin-resistant *Staphylococcus aureus* (MRSA) and West Nile virus infection in horses, and listeriosis in swine.** Targetted surveillance for MRSA in horses resulted in the increase of isolates. Other zoonotic pathogens isolated and/or identified at the AHL are given in Tables 1 & 2. These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates.

Salmonella sp., Streptococcus suis, Streptococcus zooepidemicus, and *Cryptosporidium sp.* are the most frequently identified zoonotic pathogens at the AHL since 1999. *Salmonella sp*. isolates continue to increase. In cattle and swine, *S.* Typhimurium is the most common serotype isolated and of these, the multiresistant phage type 104 is most frequent. Occupational exposure to pigs and horses is a risk factor for *S. suis* and *S. zooepidemicus* infections.

As part of our commitment to animal health surveillance, **the AHL developed new tests in 2003** such as immunohistochemistry, molecular biological tests for EEE, and IgM and IgG capture ELISA for WNV. *AHL*

Table 1. Zoonotic pathogens isolated and/or identified at the AHL, 1999 – 2003

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2003	2002	2001	2000	1999
Campylobacter coli/ jejuni/ fetus subsp. fetus	2		1	6				19	1	2	**31**	33	36	14	6
Chlamydophila psittaci				6	6					7	**19**	10	12	21	14
Coxiella burnetii (Q fever)	2			1	3						**6**	6	7	4	4
Cryptosporidium sp.	120		1		5					3	**129**	199	160	120	93
Eastern equine encephalitis			10								**10**	0	0	0	0
Francisella tularensis										1	**1**	1	3	6	0
Giardia sp.	6			1				2		1	**10**	4	19	26	4
Listeria monocytogenes	17	3		4	3						**27**	37	44	34	16
Methicillin-resistant *Staphylococcus aureus*			72							1	**73**	13	1	-	-
Mycobacterium bovis[1]											**0**	1	0	0	0
Rabies[1]											**0**	0	5	1	1
Salmonella sp.	275	139	171	6	0	51	37	2	0	143[2]	**824**	716	565	754	711
Streptococcus suis	20	360	4	3	3	0	0	0	0	2	**392**	477	380	560	489
Streptococcus equisimilis		84	27								**111**	144	126	154	93
Streptococcus zooepidemicus	2	7	192	2				2	1	5	**211**	222	175	162	220
Toxoplasma sp.				4	1						**5**	12	12	8	13
West Nile virus			3							170[3]	**173**	91			

[1] Federally reportable diseases confirmed by the Canadian Food Inspection Agency
[2] Most are from exotic species.
[3] Most are from wild birds.

Table 2. *Leptospira spp*. seropositive samples (>1:160) identified at AHL, 1999 – 2003, microscopic agglutination test (MAT)

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Feline	2003	2002	2001	2000	1999
L. autumnalis			2	120		**122**	46	32	50	2
L. bratislava		44	34	73		**151**	139	120	88	116
L .canicola			2	15		**17**	4	6		
L. grippotyphosa		13	4	59		**76**	29	44	32	18
L. hardjo	33			1		**34**	30	21	7	16
L. icterohaemorrhagiae	77	1	1	43		**122**	163	289	140	224
L. pomona	31	10	21	60		**122**	105	110	112	152
Total	**141**	**68**	**64**	**371**		**644**	**516**	**622**	**429**	**528**

AHL Lab Reports

CATTLE

2003 - Update on bovine viral diarrhea virus genotyping at the AHL

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Over the last 6 years, the AHL has identified 967 strains of bovine viral diarrhea virus (BVDV) from various bovine specimens submitted for virus isolation or RT - PCR. A detailed summary of strains by year, biotype and genotype appears in the table below.

The majority of the 921 BVDV isolates recovered in cell culture have been noncytopathic (79%), with only 21% being cytopathic.

Overall, using monoclonal antibodies or PCR genotyping, 454 (49%) strains were determined to be BVDV type 1, whereas 471 (51%) were BVDV type 2. *AHL*

Reference

Gilbert SA, et al. Typing of bovine viral diarrhea viruses directly from blood of persistently infected animals. J Clin Microbiol 1999;37:2020-2030.

Table 1. BVDV isolates by strain, biotype, and genotype, isolated at the AHL in 2003

BVDV type **Year:**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**Total**
Total strains for each year (cell culture and RT-PCR)	199	166	122	170	142	168	967
Total isolated in cell culture	199	166	122	170	136	128	921
Total cytopathic	43 (22%)[1]	33 (20%)	18 (15%)	49 (29%)	30 (22%)	25 (20%)	**198 (21%)**
Total noncytopathic	156 (78%)	133 (80%)	104 (85%)	121 (71%)	106 (78%)	103 (80%)	**723 (79%)**
BVDV type 1 cytopathic	26 (13%)	23 (14%)	7 (6%)	42 (35%)	20 (27%)	7 (14%)	125 (28%)
noncytopathic	85 (43%)	60 (36%)	35 (29%)	50 (41%)	51 (68%)	25 (49%)	306 (67%)
RT-PCR	nd	nd	nd	nd	4 (5%)	19 (37%)	23 (5%)
Total type 1	**111 (56%)**	**83 (50%)**	**42 (34%)**	**92 (54%)**	**75 (53%)**	**51 (38%)**	**454 (49%)**
BVDV type 2 cytopathic	15 (8%)	10 (6%)	11 (9%)	7 (1%)	10 (15%)	16 (19%)	69 (15%)
noncytopathic	71 (36%)	73 (44%)	69 (57%)	66 (39%)	54 (82%)	46 (55%)	379 (80%)
RT-PCR	nd	nd	nd	nd	2 (3%)	21 (25%)	23 (5%)
Total type 2	**86 (43%)**	**83 (50%)**	**80 (66%)**	**73 (43%)**	**66 (47%)**	**83 (62%)**	**471 (51%)**
BVDV type 1-2 mixture	2	0	0	1	0	0	3
BVDV untyped cytopathic	0	0	0	0	0	2 (1%)	2
noncytopathic	0	0	0	4 (2%)	1	32 (19%)	37 (4%)
Total untyped	**0**	**0**	**0**	**4 (2%)**	**1**	**34 (20%)**	**39 (4%)**

[1] proportion of the total for each year in brackets
nd = not done

Is it possible to improve case selection when considering treatment for clinical mastitis caused by *Staphylococcus aureus?*

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

Selecting the appropriate cows to treat is an important technique for increasing the success rate for mastitis treatment. Treating hopeless cases is frustrating for producers, costly, increases unwarranted antimicrobial usage, and greatly increases the risk of contamination of milk and milk products with antimicrobial residues.

While chronic udder infections with *Staphylococcus aureus* are irrefutably proven to be non-responsive to any antimicrobial therapy, **there is some evidence that neither all *S. aureus* strains nor all clinical and new infections are equally hopeless**. The number of cases that can be successfully treated may be few, but having an accurate way to identify them might be useful in targeting antimicrobial usage in herds with a high prevalence of *S. aureus* mastitis.

What are ß-lactamase-negative or ß-lactamase-positive *S. aureus*?

Beta-lactamase is an enzyme produced by some strains of *S. aureus* that destroys the ß-lactam ring of some antibiotics. This enzyme protects the microorganism against ß-lactam antibiotics such as penicillin and ampicillin. Production of ß-lactamase can be readily induced by therapy with a ß-lactam antibiotic.

Why should you test for production of ß-lactamase in *S. aureus*?

Researcher Jan Sol of the Netherlands has shown that some clinical mastitis cases caused by *S. aureus* can be cured with antimicrobial therapy. Sol reported an overall bacteriological cure rate of clinical *S. aureus* of 52%.

Three factors were associated with a higher bacteriological cure rate:

a) The cure rate was significantly higher if the SCC of the cow was low prior to the diagnosis of a case of clinical mastitis.
b) The cure rate was significantly higher if the *S. aureus* isolated was negative for the production of ß-lactamase.
c) Extending treatment beyond the labeled duration of treatment also increased the cure rate.

Infected quarters were treated 3 times at 12 h intervals. If results were unsatisfactory, treatment was continued for 2 more days.

A survey of antimicrobial susceptibility of *S. aureus* isolated from cases of bovine clinical mastitis in 11 countries in Europe and the United States, revealed that 35.6% of isolates were positive for ß-lactamase production, with an additional 21.3% becoming positive after induction by penicillin. This suggests that about 50% of isolates might be susceptible to commonly labeled antimicrobial therapies for mastitis. However, the prevalence of susceptible isolates varies from farm to farm.

Testing for production of ß-lactamase by *S. aureus* is available at the AHL

The test for the presence of ß-lactamase from S. aureus is performed using a chromogenic, cephalosporin-based (nitrocefin) disk method according to NCCLS guidelines. **A positive ß-lactamase test result predicts resistance of *S. aureus* to penicillin as well as to aminopenicillins** (ampicillin, amoxicillin), **carboxypenicillins** (carbenicillin, ticarcillin), **and ureidopenicillins** (mezlocillin, piperacillin).

Fee per test is $5. Please indicate clearly on your mastitis submission form that you would like production of ß-lactamase testing of *S. aureus*. *AHL*

References

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Osteras O, Martin SW, Edge VL. Possible risk factors associated with penicillin-resistant strains of *Staphylococcus aureus* from bovine subclinical mastitis in early lactation. J Dairy Sci 1999;82:927-938.

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard. NCCLS M31-A2, 2002;22:41.

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POULTRY

Summary of AHL pathology diagnoses for Ontario poultry, 2002-2003

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The following information is drawn from the AHL database of pathology diagnoses made at the Guelph and Kemptville laboratories, from May 2002 to September 2003.

Broiler chickens

- Over the last several years, we have diagnosed a large number of cases with **bursal atrophy**. In 2002-2003 this number increased slightly, likely due to an increased tendency to monitor the condition of the bursa. The actual diagnosis of acute infectious bursal disease (IBD) is rare. Using molecular techniques to identify and characterize IBD viruses, the AHL has identified several variant viruses associated with bursal atrophy. Other factors, such as severe septicemia, can also result in bursal atrophy and lymphoid depletion.
- **Septicemia**, primarily due to *E. coli*, is a common diagnosis. Other causes of septicemia identified include *Pseudomonas aeruginosa* and *Staphylococcus aureus. E. coli* and *P. aeruginosa* were also isolated from cases of yolk sacculitis, the latter rarely being identified as a primary pathogen. *P. aeruginosa* can also be cultured from cases of septicemia, conjunctivitis and tenosynovitis in chicks. Hatchery-derived cases have been associated with contaminated water sources or the unintentional setting of contaminated floor eggs.
- **Inclusion body hepatitis** (IBH) has been consistently diagnosed over the past few years and tends to occur in clusters. It can be related back to a common breeder flock, but multiple breeder flocks have also been identified as sources.
- **Enteritis** cases have remained steady, with coccidiosis and necrotic enteritis (*Clostridium perfringens*) being the primary diagnoses.
- A number of other diseases are consistently but less frequently diagnosed, including arthritis, rickets, spiking mortality and cellulitis.

Broiler breeder chickens

- **Bacterial septicemia**, primarily due to E. coli, was the most common diagnosis for broiler breeders from 4 days to 41 weeks of age.
- **Arthritis/tenosynovitis** is the next most common diagnosis, with *E. coli* and *Staphylococcus aureus* isolated individually or in combination.
- Less frequently, a number of other diseases are consistently diagnosed in broiler breeders, including pododermatitis, peritonitis, fatty liver-hemorrhage syndrome, urate nephrosis, and coccidial enteritis.
- Diseases that have decreased over the last few years include neoplasms (Marek's, leukosis). Myelocytomatosis is now diagnosed only infrequently.

Layer chickens

- **Osteomalacia** (cage layer fatigue) continues to be the most common diagnosis. Prolonged calcium depletion leads to softened/fractured bones and tendon ruptures.
- **Hepatic lipidosis** remains steady and tends to be an individual bird rather than a flock problem.
- **Septicemia**, primarily due to *E. coli*, is also a common diagnosis affecting birds from 5 days to 52 weeks of age.
- **Hepatitis/splenomegaly** has increased slightly and has been observed as both an individual bird and flock problem. Researchers in the US and Europe are evaluating the role of hepatitis E virus in this disease.
- Other conditions identified include **cannibalism** in 33 to 63 week old birds, and **peritonitis**.
- **Necrotic enteritis** (*C. perfringens*) was diagnosed, but has not increased over previous years.
- Four cases resembling '**peripheral neuropathy of leghorn chickens**', a disease reported at low frequency in US commercial layers, were identified in 6-12-week-old pullet flocks. Clinical signs included lameness and elevated mortality with peripheral neuritis. Unlike Marek's, the brain and spinal cord were unaffected.
- Diseases that have decreased over the last few years include neoplasms (Marek's, leukosis, other), infectious bronchitis, and coccidial enteritis.

Turkeys

- ***E. coli* septicemia** (colibacillosis) was still the number one diagnosis in turkeys; *E. coli* can also manifest as airsacculitis, yolk sacculitis, hard liver, and arthritis. The affected age group was 4 days to 9 weeks of age. *Pseudomonas aeruginosa* was also isolated from one case, a reminder that mixed infections can occur and the importance of culture and susceptibility testing to support treatment selection.
- **Mycotic pneumonia** was the next most frequent diagnosis; many of these cases had a history of straw bedding.
- **Enteritis** diagnoses, including necrotic enteritis and coccidial enteritis, remained steady.
- A number of other diseases were diagnosed (e.g., arthritis, myopathy, round heart) but were not increased over previous years.
- Disease agents that have decreased and remained low over the past several years include *Bordetella avium*, *Ornithobacterium rhinotracheale*, and *Erysipelothrix*. *AHL*

Recent cases of *Fowlpox virus, Mycoplasma gallisepticum* and *Mycoplasma synoviae*: The need for increased biosecurity awareness

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Recent global events, such as the outbreaks of highly pathogenic avian influenza in Asia and Europe, low pathogenic avian influenza in Virginia, and exotic Newcastle disease in California, highlight the importance of biosecurity at the farm level. The increasing size of farms and integration of agricultural processes increase the risks of rapid and widespread dissemination of infectious agents. Spread of these agents amongst premises can occur by a variety of modes, including unsuspected contact with contaminated birds and other animals, people, waste products, feed, water, equipment, processed products, and air-borne particles. **Three disease episodes that occurred in 2003 in Ontario highlight the necessity for constant vigilance and heightened biosecurity at the farm level.**

Over approximately 2 months, 6 commercial chicken flocks of various ages on several premises were diagnosed with **fowlpox**. Poxvirus was diagnosed by histopathology, direct electron microscopic examination of scabs, or virus isolation. Fortunately, the disease remained mild in all of these unvaccinated birds, and clinical signs disappeared in younger flocks after several weeks. An investigation into the possible links for transmission revealed a complex tangle of possibilities. The same staff working in more than one barn within a group may have spread the virus in the group. Fowlpox occurred in 2 barns that had different staff but were in geographic proximity. A farmer from a third unrelated premises experienced a fowlpox break 2 weeks after he helped move birds from one of the affected premises to a different farm. Although fowlpox occurred on his own premises, it did not develop in the barn that received the birds. A family member from the first farm to have a confirmed case also owned a separate barn in which there were a few birds with crusted heads identified at the time of shipping. These birds had been shipped prior to the first confirmed case, and they had not been tested. **Transmission of poxviruses occurs through various mechanical means.** The actions of people on these farms may have unwittingly spread the virus through contamination of their hands and clothes.

In December 2003, a positive diagnosis of ***Mycoplasma gallisepticum*** (MG) was made by serology and isolation in a large commercial flock of chickens. The flock had a 2-week period of increasing morbidity, mortalities and a minor production drop. Initial necropsy findings were consistent with *E. coli* septicemia, polyserositis and airsacculitis. Ongoing mortalities prompted further investigations. Virus isolations and serology were not significant, but MG was isolated from tracheal and sinus swabs from sick birds at the AHL. Serology follow-up on all potential contacts and tracebacks was negative. The origin of this mycoplasma infection has not been identified. Although the farm is relatively isolated from other commercial operations, there are several small backyard flocks in close proximity that may have been associated with this outbreak. Annually there are a few small backyard chicken and turkey flocks in Ontario that have serological evidence of MG and/or MS infections. MS-seropositives do occur in some commercial leghorn flocks, but **MG in large commercial flocks has been a rare occurrence**. A single MG-seropositive leghorn flock was last identified in 1994.

Mycoplasma synoviae (MS) has occasionally been identified in commercial turkeys with respiratory disease and/or tenosynovitis. **In 2003, several turkey flocks had an unusual MS infection.** The birds were seropositive to MS by serum plate agglutination and hemagglutination-inhibition testing, but the birds did not have significant clinical signs. Cultures of tracheal swabs at the AHL yielded an atypical, small, slow-growing organism that was positive for MS antigen with fluorescent antibody staining. Sequencing of the 16S rRNA gene of the AHL isolate at the University of Guelph demonstrated 98.8% identity to the MS type strain. At processing of the affected turkeys, there was no significant evidence of disease or increased condemnations.

These 3 disease episodes remind us that there are organisms present in Ontario that can create very significant diseases if they become established in commercial poultry flocks. Constant vigilance, attention to biosecurity principles, early disease investigation and laboratory testing are necessary to prevent establishment of indigenous microbes as well as catastrophic foreign animal disease organisms in our flocks. *AHL*

Avian cases with confirmed virus involvement, AHL avian virology lab, 2003

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Virus - by virus isolation, PCR, RT-PCR; poultry, wild/exotic birds; most from Ontario	# of cases	total # of pos. samples
Avian adenovirus	12	20
Avian herpesvirus	1	1
Avian influenza virus[1]	1	2
Avian paramyxovirus 1[2]	4	6
Avian reovirus	21	31
Fowlpox virus	1	1
Infectious bronchitis virus	21	27
Infectious bursal disease virus	72	98
Infectious laryngotracheitis virus	2	3
Pigeon paramyxovirus	1	1
West Nile virus	170	170

[1] Low pathogenicity H5 virus

[2] Includes vaccine viruses and a lentogenic field strain

SWINE

Rickets in a large group of 10-week old pigs

Tony van Dreumel, Gaylan Josephson, George Charbonneau, Brent Hoff *tvandreu@lsd.uoguelph.ca*

One thousand 10-week-old pigs from a 2200-head, multi-source, all-in all-out nursery were trucked to Iowa, USA. Upon arrival the next day, **200 of the 1000 pigs were down due to posterior paresis**. Within 24 hours, most of the remaining pigs showed some signs of locomotory problems. The driver had noticed some 'weak' pigs at the time of loading, but all had been ambulatory. About 150 of the smaller pigs in this age group had remained on the farm. These were examined 2 days later and 20% had some degree of locomotory problems, including dropped pasterns, front and hind leg paresis/paralysis, and knuckling of hindlimbs.

Three of these pigs were submitted live to the AHL 5 days after the shipment. These pigs had various degrees of shifting lameness, posterior paresis, body tremors, and teeth grinding. The pigs were euthanized and necropsied. The gross lesions were similar in all 3 pigs. They were small for their age, average 19.2 kg BW, but were well fleshed. All long bones were extremely soft (cut easily with a knife). The costochondral junctions were prominent, forming a so-called 'rachitic rosary' (Fig. 1). The proximal growth plates of the humeruses and femurs were moderately thickened and irregular. There was no evidence of arthritis.

Microscopic lesions in sections of ribs and long bones varied in severity, but were similar in all 3 pigs. The zones of proliferating cartilage in the costochondral junctions were markedly thickened and irregular, and contained large blood-filled cystic spaces. The zone of provisional mineralization was also highly irregular and often thickened. Irregular trabeculae with remnants of cartilage extended into the metaphysis. The trabeculae often had abundant poorly mineralized osteoid material and some were fractured. Fibroplasia was also prominent between the trabeculae. Similar lesions were present in the articular cartilage of the epiphyses of long bones. Milder but similar lesions were evident in sections of growth plates. The cortices were thin and had marked subperiosteal fibrosis.

The bone lesions were compatible with rickets. The calcium and phosphorus levels of the femurs and humeruses from the affected pigs (n = 3) were about half that of age-matched controls (n = 2). Feed samples were submitted with the pigs, but since the length of time that the affected pigs had been on the feed could not be ascertained, the analyses are not included.

Vitamin D deficiency causes rickets in young fast-growing pigs and osteomalacia in older pigs. These conditions are rarely seen now since prepared feeds contain or exceed National Research Council (NRC) recommended levels of nutrients. Deficiency problems are usually the result of feed mixing errors. *AHL*

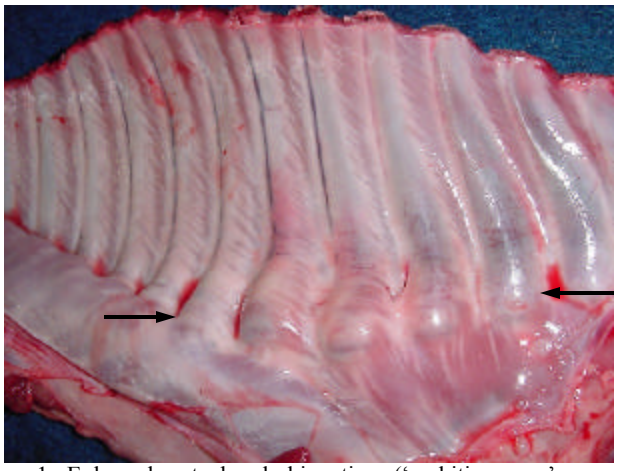

Figure 1. Enlarged costochondral junctions ('rachitic rosary', between arrows) in a 10-wk-old pig with rickets.

Update on swine influenza in Ontario

Gaylan Josephson, Susy Carman

The AHL has identified swine influenza virus in 10 herds since October, an increase over previous years. Of these, 6 isolates have been typed as H1N1 at the AHL using multiplex RT-PCR, 2 are awaiting final typing tests from the University of Wisconsin, and one very recent isolate is awaiting initial typing. In a single case, the diagnosis was made using fluorescent antibody testing, with virus isolation attempts being negative. Some of the viruses were isolated only in cell culture, some only in specific-pathogen-free embryonated eggs, while some were isolated in both.

It is important to type all strains, since pigs can act as "mixing vessels" for swine, avian and human strains of swine influenza virus. As well as traditional H1N1 swine influenza virus, wholly human H3N2 and wholly avian influenza H4N6 viruses have been identified in Ontario swine. With a dual infection in pigs, a reassortment of gene segments may occur, with the resulting virus being a combination of the 2 parental strains. Dual reassortants (H1N2) and triple reassortants (H3N2 Texas strain) can eventually occur as recently happened in swine in the Midwestern USA, to produce a virus with swine, avian and human genes. Reassortant H1N1 are also present in swine in the USA. **There is potential for direct transmission of a new reassortant virus from pigs to man.** This accounts for the sudden emergence of some antigenically different strains of influenza virus in man in recent years. *AHL*

Lawsonia intracellularis PCR test evaluated and validated

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Current methods for the diagnosis of *Lawsonia intracellularis* infection have limitations. Traditional methods have relied on post-mortem examination, with histopathology and the use of special stains to detect these intracellular organisms. Diagnosis in live pigs has been limited to serological methods or examination of fecal smears for the presence of *L. intracellularis* organisms.

We tested 100 tissue samples (ileal or occasionally colonic scrapings), obtained from diagnostic samples submitted to the AHL, by PCR for the presence of *L. intracellularis* organisms. Corresponding specimens were examined histologically, using a Warthin-Starry silver stain to confirm the presence of organisms in the apical cytoplasm of proliferative crypt cells. Fecal smears were stained with a modified acid-fast (MAF) stain and examined for the presence of typical organisms.

We tested 86 fecal samples by PCR for the presence of *L. intracellularis* organisms. As few as 10 *L. intracellularis* organisms can be detected by PCR in DNA extracted from infected mucosal infiltrate, so the PCR test is extremely sensitive. **The sensitivity of the PCR test on tissue samples was 95.5%, with a specificity of 90.9%. The sensitivity of the PCR test on feces was 85.7%, with a specificity of 78.4%.**

Previous reports indicate a lower sensitivity and specificity for fecal testing, due in part to the presence of many inhibitory factors within feces. Our values may be higher because most specimens were submitted when the animal was in the acute phase of the disease, or had died from the disease, and potentially had large numbers of organisms present. Examination of MAF fecal smears for the detection of *L. intracellularis* organisms has low sensitivity. Since this test was one of the comparison tests used in this project, the low fecal specificity of PCR is easily explained. In addition, our calculations were made at the individual animal level. Calculations made at the herd level would result in higher sensitivity.

The AHL is now offering the PCR test for *L. intracellularis* for diagnostic purposes at a fee of $16 per test. We recommend that you submit several fecal samples, and use the test as a herd test, rather than as an individual animal test.

This project was funded by Ontario Pork and Pharmacia Animal Health. *AHL*

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SMALL RUMINANTS

An outbreak of *Clostridium perfringens* type D enterotoxemia in milking does

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Three recently purchased milking does (two Saanen and one Alpine) were submitted for necropsy with a history of sudden death of 9 of 325 does (3%) in a milking flock. At postmortem, all does were obese. Significant lesions included mild to marked pulmonary edema and congestion in all 3 does, and severe colonic mucosal necrosis in 1 doe. Histologically, marked pulmonary edema was the only significant lesion present in 2 does, and the third doe had marked multifocal necrohemorrhagic colitis with intralesional colonization of clostridial-like bacteria. Bacterial culture of small intestine and/or colon yielded a heavy pure growth of *Clostridium perfringens.*

A diagnosis of *C. perfringens* type D enterotoxemia was confirmed by the detection of epsilon toxin typical of *C. perfringens* type D on genotyping, the gold standard for diagnosis. Almost 90% of the affected does in this outbreak were recently purchased, and they had not been vaccinated for *C. perfringens* type D at their home farm.

***C. perfringens* type D enterotoxemia should be considered in the differential diagnosis of sudden death in small ruminants.** The most striking postmortem findings in goats naturally infected with *C. perfringens* type D consist of pulmonary edema and necrotizing pseudomembranous colitis. **Colitis can be missed easily in autolysed cases, and pulmonary edema may be the only lesion observed**. Cerebral vasogenic edema or bilaterally symmetrical encephalomalacia, the pathognomonic lesion of *C. perfringens* type D in sheep, is not a common lesion in goats naturally infected with *C. perfringens* type D. *AHL*

Arthrogryposis in lambs – have you seen any cases?

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Within a 2-week period this winter, the AHL received multiple specimens from 3 flocks that had stillborn lambs with severe arthrogryposis. The flocks were in different areas in the province and represented Suffolk, Dorset, and East Friesian breeds. Gross lesions in lambs submitted for necropsy included arthrogryposis and hydranencephaly (Figure 1). Chondrodysplasia was present in one flock, whereas scoliosis, kyphosis and torticollis were present in 2 lambs from another flock. Additional histological lesions included dysplasia of the spinal cord with hypoplasia of gray matter, hypoplasia of skeletal muscle myofibers and follicular dysplasia in sections of skin.

Rule-outs include a myriad of viral infections, toxins and genetic abnormalities. Border disease virus (BDV), Cache Valley virus, and several other bunyaviruses endemic to North America have been shown to be capable of causing arthrogryposis and brain malformations when dams are exposed in early pregnancy. Cache Valley virus and snowshoe hare virus, both bunyaviruses, have been previously identified in Ontario. **Infection induces an antibody response in the fetus, so it is rare to be able to isolate virus at the time of birth. To diagnose infection with these viruses, it is important that the practitioner collect precolostral serum from lambs (preferred) or serum from ewes.**

Plant toxins include locoweed (*Oxytropis*), *Astragalus*, *Veratrum californicum*, tobacco, lupins and several species of hemlocks. Parbendazole given to ewes at 12-24 days gestation produces similar lesions. Ovine hereditary chondrodysplasia - also known as "spider lamb syndrome", a genetic disease of sheep that produces musculoskeletal deformities - has occurred in Ontario.

Test results are pending on this current series of cases and we will update you with significant findings in a future edition of the newsletter. In the interim, if you have had flocks with similar lesions, please contact the AHL.

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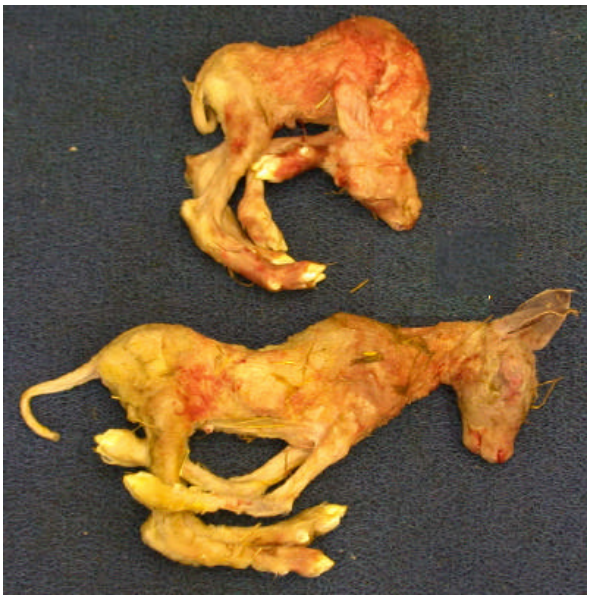

Figure 1. Arthrogrypotic ovine fetuses.

HORSES

Ontario Racing Commission Death Registry

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As many practitioners know, the Ontario Racing Committee (ORC) now requires necropsy examinations on all racing or recently qualified horses that die or are euthanized. During 2003, 125 horses were necropsied at the AHL through the ORC Death Registry. The information obtained from these examinations provides excellent baseline data on equine health in Ontario and will be invaluable in contributing to research in equine medicine and surgery. This program will continue during 2004.

The ORC is currently preparing a report of Death Registry cases for the past year and results will be presented soon to stakeholders. A summary of this information will be available in the June 2004 AHL Newsletter – stay tuned!

AHL

West Nile virus infection in a pony in eastern Ontario

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On October 10, 2003, a 21 yr-old gray pony mare from a small private stable in eastern Ontario was euthanized and presented for necropsy to AHL-Kemptville. The pony was reported by the veterinarian to have been fully vaccinated for WNV using a killed vaccine in the fall of 2002, and had been given annual booster vaccines for eastern equine encephalitis (EEE), western equine encephalitis (WEE), and West Nile virus (WNV) in April of 2003.

Thirty-three days prior to euthanasia, the pony had shown stumbling, mild 4-limb ataxia that was worse on the hind legs, an unusual lateral head-swinging movement, and reluctance to leave the stall. Her body temperature, appetite, attitude and cranial nerve function were reported as normal. Results of a CBC and biochemical profile were unremarkable. Serum was tested for WNV IgM antibody using the antibody capture ELISA, and results were negative. Results of serological testing showed a stable acute and convalescent serum titer of 1:80 for antibodies to EEE and of 1:20 to WEE. The pony was treated with intravenous flunixin meglumine (Banamine) for 3 days, after which time some improvement in strength and other clinical signs was observed, including disappearance of the head swinging.

This case presented a diagnostic challenge and typified the variable clinical presentation of WNV encephalomyelitis

The pony was re-examined 2½ weeks later due to a marked sudden worsening of clinical signs, including 4-limb asymmetrical ataxia (worse on the left), weakness, dragging of all 4 feet, lateral head swinging and a splay-legged posture during grazing. Treatment with corticosteroid drugs was initiated, but was discontinued after 24 hours because of the onset of severe sweating that continued for several days. On examination 3 days later, progression of clinical signs was observed, and unilateral atrophy of the left gluteal muscles had become obvious. The pony would collapse when the upper left hindlimb was touched, and the owner elected euthanasia a few days later due to safety concerns.

At necropsy, there were no gross lesions of the brain or spinal cord segments taken from proximal cervical, distal thoracic and proximal lumbar areas. Histopathology consisted of severe nonsuppurative encephalomyelitis characterized by multifocal gliosis, lymphocytic perivascular cuffs, digestion chambers adjacent to areas of inflammation, and non-suppurative spinal meningitis. The brain was tested for rabies by fluorescent antibody test and tissue culture, for EEE by real-time PCR, and for *Sarcocystis neurona* by immunohistochemistry, with negative results. However, **brain tested positive by real-time PCR for WNV.**

This case presented a diagnostic challenge and typified the variable clinical presentation of WNV encephalomyelitis, including a relatively long clinical course compared to many reported cases, and recurrence of signs after initial partial abatement. Also, although some horses develop WNV disease after receiving only a single dose of killed vaccine, disease is reported to be less common in horses receiving 2 or more doses of the vaccine. It is possible that this pony did not mount a normal immune response to vaccination due to immune-suppression, or that immunity had waned between the early April vaccination date and the viral challenge 4 months later. This case serves as a reminder that IgM antibodies may not always be detected in a single serum sample from horses early post-infection; **it is recommended that when the initial IgM test is negative but clinical signs suggest WNV disease, a second sample be submitted.** For horses that survive WNV disease, testing acute and convalescent sera for the presence of virus neutralizing antibodies should also be requested. *AHL*

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Companion animals - New *Giardia* ELISA - *continued from page 12*

Recently, a number of commercial ELISA's have been developed that allow detection of *Giardia*-specific antigen in feces. The ProSpecT® *Giardia* Microplate Assay (Alexon Trend) is one of these ELISA's, and we now offer this test for dogs as it appears to have at least the same level of sensitivity and specificity as zinc sulfate flotation for *Giardia* cysts. The method uses a monoclonal antibody for detection of GSA 65 (a parasite-specific antigen), which is released by trophozoites as they multiply within the host's intestinal tract.

Ideally, fresh fecal samples should be examined that have been stored at 2-8°C and tested within 48 hours of collection. If this is not possible, samples can be frozen at –20°C or less and submitted frozen. Alternatively, fecal specimens can be treated with 10% formalin or SAF fixative, refrigerated or stored at room temperature, then tested within 2 months of collection. The AHL will examine samples once a week, on Fridays (turnaround time of ≤7 d), and the cost per sample is $30.00. *AHL*

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COMPANION ANIMALS

Pug dog encephalitis

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A unique encephalitis of Pug dogs was first recognized in the 1960's in California. Originally described in the veterinary literature in 1983, Pug dog encephalitis (PDE) has been reported in the United States, Australia, New Zealand, Japan and Europe. PDE usually occurs in juvenile to young-adult dogs, although it has been reported in a 7-year-old dog. Seizures are the usual presenting sign, although circling, head pressing, and blindness may also occur. Some dogs will die or are euthanized within days of clinical onset, whereas others may continue for months with intermittent seizures, depression, or visual disturbances. It has been described in littermates and closely related dogs, however, it also occurs sporadically.

Since 1998, Ontario Veterinary College (OVC) and AHL pathologists have diagnosed PDE in 6 dogs. Three males and 3 females were affected; the ages given for only two of the dogs were 10 months and 1.5 years. Three of the 4 dogs that were presented with seizures died or were euthanized within days to weeks of onset of clinical signs, whereas one dog had intermittent seizures over a 9-month course. An acute episode of circling with a head tilt was observed in one dog, and another was described as having neurological signs.

Histologically, all dogs had necrotizing nonsuppurative meningoencephalitis primarily affecting the gray and white matter of the cerebral cortices (Figure 1). Granulomatous meningoencephalitis (GME) can be difficult to differentiate from PDE. The key histological distinction is that the necrotizing nonsuppurative lesions in GME are predominantly within white matter. As with PDE, the cause of GME is unknown.

The cause of PDE is not known and it is invariably fatal. PDE should be included in the list of differential diagnoses of infectious, toxic and other inflammatory diseases of the nervous system, particularly in young Pugs that have been presented with seizures. *AHL*

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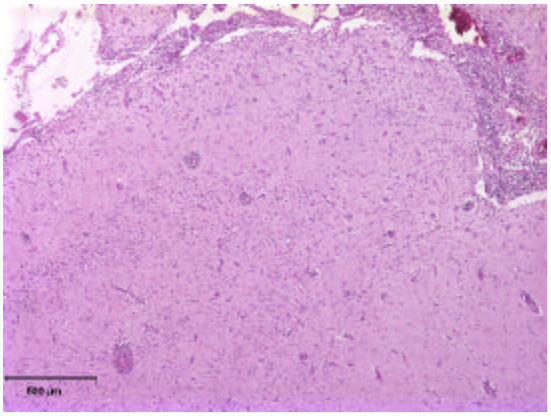

Figure 1. Nonsuppurative meningoencephalitis in a Pug.

New *Giardia* ELISA in the parasitology lab

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Two recent studies have demonstrated that 7.8%-9.9% of dogs visiting suburban/urban practices in southern Ontario are infected with *Giardia*. Interestingly, at least 78% of the *Giardia*-positive dogs in these studies had subclinical infections. In addition, animals less than a year of age were much more likely to be infected than older animals.

In light of the zoonotic potential of canine *Giardia* infections, and the fact that the prevalence of *Giardia* in Ontario dogs is at least twice the prevalence of *Toxocara canis* in the same population of animals, it is prudent to regularly screen dogs for *Giardia*. This is particularly important for both young animals and dogs that reside in households with either young children or immunocompromised individuals.

Giardia infections have traditionally been diagnosed by examining feces for trophozoites or cysts of the parasite. However, many people have problems identifying these structures. Furthermore, they are often present in low numbers. *(continued on page 11)*