UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

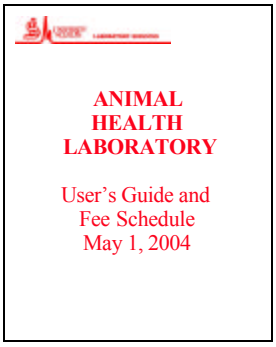
LAB REPORTS

May 1, 2004, AHL User's Guide and Fee Schedule

In addition to sample requirements, usual testing schedules, and expected turnaround times, we have incorporated 'Common disease conditions', clinical pathology profile interpretation, and reference interval tables in this edition. **We hope that this combined source of information will save you time and help you to make the most of our services**. We welcome your comments about the new format. The Guide/Schedule is also available on the Web at http://www.uoguelph.ca/ahl/FeeSchedule/AHLFees.html .

Significant changes in the May 1, 2004, User's Guide and Fee Schedule include:

- Adjustments to some of our fees to reflect increasing costs of materials; added new tests to the test menu and deleted some tests that have been used infrequently or that have become outmoded.
- Added various new molecular-based (PCR) tests in avian virology, mammalian virology, and bacteriology.
- Changed some of the metals tests in Toxicology to 'Mineral panels' to allow more comprehensive testing.
- Added new immunohistochemistry tests in Pathology, including tests for additional infectious agents and tumor markers.



We will continue to strive to provide the most cost-effective testing strategies to help you deal with animal health challenges, and will keep you posted on changes in test availability and fees through our quarterly AHL Newsletter, which is also on the Web at http://www.uoguelph.ca/ahl/NewsletterLink/ahlnewstable2.html .

Quick tips:

- Samples arriving in **Bacteriology** before 1800 hr will be set up for culture that day. Samples arriving after 1800 may not be set up until the next business day.
- Our new ***Lawsonia intracellularis* PCR test** is validated and available for clients. The test is done on feces from live animals, or intestinal mucosa (ileum preferred) from dead animals. The fee is $16/test for food animals, and $32/test for non-food animals.
- After its peak of activity in 2002, **West Nile virus** is likely to be present at a low level as in the summer of 2003. Up-to-date information on WNV status can be obtained through our website at http://www.uoguelph.ca/ahl/WestNileInquiries/WestNile.htm .
- We offer **scrapie-resistance PrP genotyping** for codons 136, 154, 171. We require 5 mL of EDTA blood, and the fee is $60.
- **PCR for swine influenza virus** and **IgG IFA for PRRSV** are now available.
- We are currently finishing the validation of **real-time PCR testing for *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*** - testing is currently free of charge. These tests will be available in June 2004.

Laboratory response to an animal health emergency

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What would the AHL do in the event of a major animal health emergency in Ontario?

- highly pathogenic avian influenza (HPAI)
- foot-and-mouth disease (FMD)
- exotic Newcastle disease (END)
- other foreign animal diseases (FAD's)
- other emergency event

Given the intensity of livestock and poultry production in Ontario, and the speed of modern transportation systems, there is great potential for spread of pathogens that could wreak havoc on this vital industry.

Suspected FAD's must be reported immediately to the Canadian Food Inspection Agency (CFIA), which has primary responsibility for FAD diagnosis, control, and eradication. Diagnosticians at the AHL are vigilant regarding the detection of FAD's, such as FMD, and zoonotic diseases, such as anthrax, and several have had advanced training in recognition of FAD's at the CFIA's National Center for Foreign Animal Disease in Winnipeg.

As part of the **Transmissible Spongiform Encephalopathy (TSE) Network** organized by CFIA, and with financial support from the Ontario Ministry of Agriculture and Food (OMAF), the AHL has been doing surveillance testing by immunohistochemistry for bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD) in Ontario - more than 3000 BSE tests and more than 1300 CWD tests over the past 2 years. CFIA has provided member labs with standard operating procedures (SOP's) and proficiency panels, and this alliance is a splendid model for other partnerships.

Similarly, we have harmonized SOP's with CFIA for agar gel immunodiffusion testing for avian influenza virus (AIV) antibodies. We also have a validated PCR test in-house for AIV. Given the experience of Virginia with LPAI and of California with END, federal resources can rapidly be stretched to the limit in the face of a major outbreak, and state/provincial resources need to be at the ready. However, the AHL has limited surge capacity, and routine services could be severely disrupted in the event of a major new testing demand.

The AHL is a member of the **Canadian Animal Health Laboratorians Network** (CAHLN), a body put in place in order to share information on animal health diagnostic trends, techniques and research; to provide a venue for "networking" to identify common issues of concern; and to facilitate linkages among organizations and scientific staff involved in animal health diagnostic work in Canada. The theme of the annual meeting of CAHLN being held in Guelph this year, May 16-19, is "Emergency preparedness", and we hope that this and other ongoing activities will contribute to an enhanced state of readiness for the lab. *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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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contributed to the generation of results reported in the AHL Newsletter.

Strategic use of laboratory testing in trying times

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The diagnosis of BSE in Canada has had a tremendous impact on not only the beef industry but also the dairy industry. **In the dairy sector, the poor cull cow market and the loss of heifer exports have caused cow numbers to swell on many farms**. Barring the purchase of additional quota, the pressure to keep production within quota limits is forcing many producers to re-evaluate their cow inventory and consider reducing herd size to pre-May 20, 2003, numbers.

For many herds, this situation presents an opportunity to reduce the prevalence of certain production-limiting diseases. Three conditions to consider are *Staphylococcus aureus* intramammary infection, *Neospora* infections, and Johne's disease.

Staphylococcus aureus

Most practitioners are familiar with the cyclic shedding patterns of *S. aureus* (SA) and know that repeated culturing and the freezing of milk samples prior to culture may enhance the sensitivity of detection. But once a culture-positive cow is identified, there are a number of factors to consider before making a recommendation to cull the infected cow. Recent work indicates that SA cure depends on the age of the cow, linear score at drying off, the quarter involved, the rate of shedding of SA, and the frequency of culture. Younger cows are more likely to cure than older cows; infected cows with higher linear scores (>5) at dry-off are less likely to cure; rear quarters are less likely to cure than front quarters; and cows that have fewer organisms recovered at culture are more likely to cure. Cows that have had only 1 positive SA culture out of 3 cultures prior to dry-off are more likely to cure than cows with multiple positive cultures. It is generally believed that the more chronic SA infections are less likely to cure, therefore a higher cure rate for younger cows with lower linear scores makes sense. No one is quite sure what the quarter location (front versus rear) effect means, however it has been reported in other studies as well. The last 2 factors may be related to the epidemiologic specificity of the test. One positive SA culture with low numbers of organisms may be a false-positive diagnosis and may not be a true intramammary infection. **Select your cows wisely.**

> The current situation presents an opportunity to reduce the prevalence of certain production-limiting diseases.

Neospora caninum

A frequently quoted study from California suggests that there is increased culling risk and milk production loss associated with *N. caninum* infection. However, recent work in Ontario suggests that infection does NOT increase the risk of culling in most herds, and there is no effect of infection on milk production of cows in herds not experiencing abortion problems. However, where abortion is a problem and where *N. caninum* is a contributing cause of abortion, there is evidence of reduced milk production and increased culling risk associated with *N. caninum* infection. The bottom line: **consider culling *N. caninum*-infected cows only in herds experiencing abortion problems.**

Johne's disease *(Mycobacterium avium subsp. paratuberculosis, Map)*

Mycobacterium paratuberculosis infection is more common in Ontario dairy herds than previously estimated. A study completed in 2003 found that 20% of herds had 2 or more cows test positive on a Johne's milk ELISA (Hendrick et al., unpublished). This means that producers who recently expanded their herd had a 1 in 4 chance of purchasing animals from an infected herd. As veterinarians, we should use our previous knowledge of a farm to decide if the cows on that farm are at increased risk for Johne's disease. History tells us that farms where Johne's disease has previously been diagnosed or replacement animals purchased, are more likely to have Johne's disease present in their herd today. **Selecting cows to be culled for Johne's disease is best done by first screening every cow in the herd with a milk or serum ELISA and then confirming all ELISA-positive cows using a fecal test.** Since last fall, the AHL has offered 2 new Johne's fecal tests to complement the serum ELISA: a direct fecal PCR test, and BACTEC fecal culture. The fecal PCR test identifies a sequence of DNA from *Map* that is present in the manure of shedding cattle. This PCR test can be completed in as little as 3 days and costs $22.50 per sample, but it is not as sensitive as the BACTEC fecal culture, which will find twice as many infected cows as the fecal PCR test. The BACTEC culture ($27.50/sample) utilizes a liquid medium that is capable of growing the bacterium in as little as 3 weeks for cows shedding large numbers of organisms. **The BACTEC fecal culture is currently the best method for confirming the Johne's infection status of cattle.** *AHL*

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AHL Lab Reports

RUMINANTS

Diagnoses of calf diarrhea, 2003

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In 2003, 181 cases from calves <3 months old with gastrointestinal disease were submitted to AHL pathology. The mean age of calves was 20 days (range 1-90 days) and the median age was 10 days. Most were dairy (n = 110) or beef (n = 52) calves, a few were veal calves (n = 8), and the rest (n = 11) were identified only as cattle.

Enteritis/enterocolitis comprised over 82% (n = 162) of the diagnoses. The remainder were abomasitis (n = 8), abomasal bloat (n = 7), rumenitis (n = 6), intestinal accidents (n = 6) and occasional cases of ruminal bloat, abomasal ulcers, esophagitis, glossitis or anomalies.

A single pathogen was identified or isolated in 58% of enteritis/colitis cases, although multiple pathogens occurred in 22% of cases; etiology was not determined in 20% of cases. The pathogens identified or isolated are given in Table 1.

Multifactorial causes of enteritis often included *Cryptosporidium sp* or *E. coli* (Table 2). Reaching an etiologic diagnosis in cases of scouring calves depends on the agent(s) involved, duration of infection, samples submitted, and range of tests requested. **Cases in which calves were submitted for necropsy were more than twice as likely to yield an etiologic diagnosis compared to cases where samples were chosen in the field.** This reflects the panel of bacterial and virological tests routinely used to evaluate necropsy cases at the AHL. **For field cases, sampling several sections of small and large intestine, and freezing and fixing tissues in formalin (immediately after euthanasia) could increase etiologic diagnoses.** When budget is an issue, request histopathology first and let the pathologist decide on appropriate microbiological tests for the frozen tissues based on the lesions. *AHL*

Table 1. Pathogens identified or isolated from calves with enteritis, AHL pathology, 2003

Pathogen	Number identified
Escherichia coli	42 (27%)
Cryptosporidium sp.	34 (22%)
BVDV	23 (15%)
Coronavirus	18 (11%)
Rotavirus	16 (10%)
Salmonella sp	10 (6%)
Bacteria (not specified)	5 (3%)
Coccidia	4 (3%)
Clostridium perfringens type A	2 (1%)
Adenovirus	1 (1%)
Clostridium sp.	1 (1%)
Parasitic	1 (1%)
Total	157

Table 2. Multifactorial causes of calf enteritis, AHL pathology, 2003

Pathogen 1	Pathogen 2	Pathogen 3	Number of cases of enteritis
Cryptosporidium sp.	BVDV	-	6
Cryptosporidium sp.	*E. coli*	-	6
Cryptosporidium sp.	Coronavirus	-	4
Cryptosporidium sp.	Rotavirus	-	4
E. coli	*Salmonella sp.*	-	2
E. coli	*Cryptosporidium sp.*	Coronavirus	2
E. coli	Rotavirus	Coronavirus	2
Cryptosporidium sp.	*Salmonella* sp	-	1
E. coli	Rotavirus	-	1
E. coli	BVDV	-	1
E. coli	*C. perfringens* type A	-	1
Coronavirus	Rotavirus	-	1
E. coli	*Cryptosporidium sp*	BVDV	1

Update on BVD virus testing - BVD virus type 1b serology is now available at the AHL

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The AHL offers a complete range of virus detection and serological tests for the detection of bovine viral diarrhea virus (BVDV) infection.

- For BVDV detection and herd screening, the AHL offers antigen detection BVDV ELISA, PCR, and virus isolation. **Tests are selected according to the individual needs of each herd**.
- We also offer virus neutralization (VN) testing, necropsy, histopathology, and immunohistochemistry to assist with the diagnosis of BVDV infection.

These various testing methods and recommendations for their best use are reviewed in our **BVD virus LabNote**, which has recently been updated and posted on our website - http://ahl.uoguelph.ca ,click on "Newsletters, LabNotes", go to the bottom of the page and click on "BVD testing – updated Mar 16, 2004". You will be able to print this file.

BVD virus can now be divided into type 1a, 1b, 2a and 2b genotypes, depending on their genetic sequences. This genetic variation results in differences in viral proteins. These differences are reflected in antibody titers in cross-neutralization assays, such that antibody titers are higher to the genotype of virus causing the infection. As a consequence, both BVDV type 1 and 2 viruses are now included in many BVDV vaccines.

- The AHL routinely offers virus neutralization antibody assays for BVDV type 1a (Singer, NADL) and BVDV type 2a (NVSL 125c).
- **We also now offer virus neutralization antibody assays for determination of antibody titers to BVDV type 1b, using the virus strain called TGAC.** *AHL*

Ovine abortion diagnoses, 1998 - 2004

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Causes of ovine abortions have not changed substantially over the past 6 years (Table 1). Of these, 44 (16%) were *Toxoplasma sp*., 37 (13%) were *Chlamydophila abortus* (formerly *Chlamydia psittaci*), 10 (4%) were *Campylobacter jejuni,* and 7 (2%) were *Coxiella burnetii* abortions.

Several of these agents can cause **serious infections in people** and care must be taken when handling and packing sheep and goat abortion specimens. **Pregnant women should not handle tissues from sheep or goat abortions.**

The chance of obtaining an etiologic diagnosis greatly increases with appropriate sampling. Because **diagnostic lesions are more frequent in small ruminant placentas than in fetuses,** it is very important to submit placenta as well as brain, parenchymal organs, skeletal muscle and intestines for histopathology and selected tissues for microbiology. (See the new AHL User's Guide and Fee Schedule for details). *AHL*

Table 1. Pathology diagnoses in ovine abortion cases, March 1998 – March 2004

Agent/condition	98/99	99/00	00/01	01/02	02/03	03/04	Total
Idiopathic	21	37	16	15	8	12	109
Toxoplasma sp	12	4	13	9	2	4	44
Chlamydophila abortus	8	9	7	4	6	3	37
Placentitis	1	6	4	5	3	5	24
Stillbirth	3	0	8	2	3	1	17
Bacteria (miscellaneous)	0	4	5	3	3	1	16
Campylobacter jejuni	0	2	2	1	3	2	10
Infectious, etiology unknown	5	2	1	0	0	0	8
Coxiella burnetii (Q fever)	1	1	1	3	1	0	7
Other (mummified fetus/anomalies)	4	0	0	0	0	3	7
Bacillus licheniformis	1	0	0	0	0	0	1
Salmonella Arizonae	0	1	0	0	0	0	1
Salmonella Tennessee	1	0	0	0	0	0	1
TOTAL	**57**	**66**	**57**	**42**	**29**	**31**	**282**

POULTRY

Laboratory diagnosis of avian influenza infection

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Avian influenza (AI) is a highly contagious viral disease caused by type A influenza virus that affects a wide range of bird species including food-producing birds, wild birds and pet birds. Two forms of the virus are recognized based on their pathogenic effects on chickens. Low pathogenic AI (LPAI) virus can cause mortalities slightly above normal, whereas highly pathogenic AI (HPAI) virus can cause mortalities up to 100% over several days.

The clinical signs of the disease can be quite variable, non-specific, and similar to several other diseases.
LPAI can have:

- Many affected birds with few deaths
- Respiratory signs – rales, sneezing, coughing
- Decreased egg production
- Ruffled feathers, depression
- Decreased activity, quiet birds
- Decreased feed and water consumption
- Diarrhea

HPAI can have:

- Many affected birds with many deaths
- Sudden death with no signs
- Neurological signs – tremors, inability to stand
- Severe depression, quiet birds
- Respiratory signs – rales, sneezing, coughing
- Sudden, severe drop in egg production with abnormal eggs
- Swollen congested combs and wattles, swelling around the eyes
- Hemorrhages on the legs

Clinical signs can be suggestive of AI, but laboratory testing is essential to confirm the diagnosis. **The AHL offers several diagnostic tests for avian influenza virus.** A laboratory diagnosis of avian influenza virus (AIV) infection can be established by (1) demonstrating the virus by (i) isolation, (ii) detection of AIV genetic material, (iii) detection of AIV proteins and/or (2) detection of antibodies against AIV.

Virus isolation is a very sensitive technique for the diagnosis of AIV infection, provided that specimens are of good quality. Actually, **virus isolation in embryonated eggs along with subsequent confirmation by molecular or immunological methods is still the gold standard for the diagnosis of AIV infection.** A valuable feature of virus isolation is that the virus is available for further characterization if necessary. A disadvantage of virus isolation is that it will take days, or even weeks, before final results are available.

The detection of AIV genetic material by **real-time RT-PCR** is now routinely used for the diagnosis of AIV infection. The advantage of this molecular method, which is comparable in its sensitivity to virus isolation, is that the results can be obtained within 24 hrs when requested.

The detection of viral proteins in animal samples using **human antigen-capture kits** has also been successfully utilized in veterinary medicine. These kits are very rapid and can provide results within hours, but their sensitivity and specificity are somewhat lower when compared to virus isolation and RT-PCR.

The presence of antibodies to AIV is routinely detected by the **agar gel immunodiffusion (AGID) test**. Although this test has been routinely used to detect antibodies to AIV in chickens and turkeys, there is considerable variation in levels of precipitating antibodies produced in other avian species. Therefore, although AGID is a gold standard for AIV serology in chickens and turkeys, results for other avian species must be interpreted cautiously.

It is important to remember that the success of laboratory diagnosis is impacted by the type and quality of collected specimens and conditions for storing and transporting the specimens to the laboratory. Avian influenza viruses can be readily detected from tracheal or cloacal swabs or tissue samples from respiratory and intestinal tracts. Highly pathogenic viruses cause systemic infection and virtually any organ can be used for virus detection. Specimens should be chilled or frozen immediately after collection and shipped on ice packs to the laboratory. If specimens cannot reach the laboratory within 48 hours, they should be kept frozen. Repeated freezing and thawing should be avoided to prevent the loss of infectivity. Samples for virus isolation should not be stored or shipped in dry ice unless they are sealed in glass or plastic (double-bagged) since CO_2 can inactivate influenza viruses if it reaches the sample during transport. Serum samples for AGID can be kept in the fridge for 1 week, but should be frozen for long-term storage.

The confirmation of suspected cases of AI and pathogenicity testing are performed at the National Centre for Foreign Animal Diseases in Winnipeg.

In suspected cases of AI, producers should immediately contact a poultry veterinarian, institute strict biosecurity and isolation procedures, and contact CFIA. Local CFIA district offices are in the blue pages of your local phone directory

Contact for Ontario is CFIA, Guelph 519-837-9400
CFIA Foreign Animal Disease Hotline 1-877-814-2342
Avian influenza information is on the CFIA website http://www.inspection.gc.ca/english/anima/heasan/disemala/avflu/avflue.shtml *AHL*

SWINE

A confined outbreak of encephalomyelitis in a multi-aged group of pigs

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Over a 2-week period in early November 2003, three sows, three 13-week-old piglets, and four 17-week-old pigs, from a 1700-sow farrow-to-finish multi-site operation, were submitted to the AHL for postmortem examination with a history of sudden death and/or acute nervous signs (paddling, tremors, and lateral recumbency). No other animals in this operation exhibited similar clinical signs, and there was no history of a concomitant disease process. The gross lesions noted were inconsistent and variable, and included fibrinous polyserositis (1 piglet), bronchopneumonia or bronchointerstitial pneumonia (2 piglets), and gastric ulcers (1 piglet). No significant gross lesions were noted in the central nervous system (CNS).

Histological lesions in the CNS in 8 of 10 animals were similar but varied in severity and consisted of nonsuppurative meningoencephalomyelitis with a few eosinophils in lymphoplasmacytic cuffs, multifocal glial nodules (focal gliosis), and occasional satellitosis and neuronophagia (Figure 1). The distribution of lesions was mainly in, but not restricted to, the grey matter of brain stem and spinal cord. Based on these results, **a tentative diagnosis of viral encephalomyelitis was made**. Agents capable of causing porcine viral encephalomyelitis include porcine herpesvirus 1 (pseudorabies), rabies virus, hemagglutinating encephalomyelitis virus (HEV), porcine enterovirus, PRRSV, classical swine fever virus, African swine fever virus, or porcine paramyxovirus.

Rabies and pseudorabies were ruled out by immunofluorescence and/or isolation by the Canadian Food Inspection Agency. At the AHL, virus isolation using primary pig kidney, primary pig thyroid, swine testes, VERO, baby hamster kidney-21, and MARC-145 cells failed to identify any viruses in various tissues from the 10 animals, including brain from the younger piglets and sows. Immunohistochemistry (IHC) using antibody to human enterovirus did not demonstrate viral antigen in multiple brain sections from the 2 younger piglets. A field strain of PRRSV with RFLP type 124 was identified in homogenates of lung, tonsil, and lymph node from all 17-week-old pigs, although brain from these pigs was negative for PRRSV antigen by IHC, and PRRSV-PCR was also negative using brain tissue from this group. IHC for porcine circovirus 2 (PCV-2) was strongly positive in foci of inflammation in brain and spinal cord of 7 animals tested from all age groups, but PCV-2 nucleic acid was not identified in brain of any of the 3 younger piglets and 3 sows tested by PCR. Bacteria isolated from meningeal swabs included *Streptococcus suis* (2 piglets), *Streptococcus equisimilis* (1 piglet, isolated in association with *S. suis*), and *Actinobacillus pleuropneumoniae* serotype 6(8) (1 sow). Sera taken from additional nursery and finisher pigs 1 week after the last clinical case were tested for the presence of HEV antibody. HI titers in nursery pigs ranged from <1:8 to 1:512, whereas titers in the finisher pigs ranged from <1:8 to 1:32, suggesting that HEV infection was present in the sow herd and may have been of clinical importance.

This unusual porcine neurologic outbreak may have been due to a combination of PRRSV immunosuppression, PCV-2 infection, and secondary bacterial infection. On the basis of serology results, HEV infection cannot be excluded, but titer determination on paired serum samples would be required to confirm this. To the best of our knowledge, encephalitis has not been previously associated with natural infection with PCV-2. PCV-2 antigen has been identified in brain of pigs with naturally occurring congenital tremors, although association of the virus with this clinical condition remains unclear. PCV-2 antigen has been identified in brain after virus injection into cesarean-derived, colostrum-deprived piglets, demonstrating neurotropism of the virus under experimental conditions. PRRSV may have influenced clinical disease in some of the pigs in our case, and although infection with a field strain of PRRSV was demonstrated only in the 17-week-old pigs and there were no other clinical signs of PRRSV infection in the herd at the time, this herd was serologically positive for PRRSV. *AHL*

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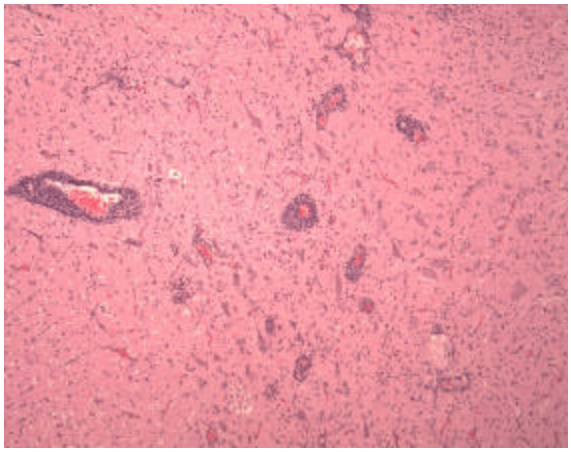

Fig. 1. Spinal cord of a piglet with severe lymphoplasmacytic cuffs and patchy focal gliosis.

AHL swine disease summary, 2003

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While the number of swine submissions to the AHL increased in 2003 over 2002 by 5.9% (2636 vs. 2488), the number of submissions consisting of carcasses decreased from 375 to 329 (12.3%) and the number of submissions that contained formalinized tissues for histological examination decreased from 376 to 305 (18.9%). The decrease in pathology-related submissions was more than offset by an increase in the number of submissions in which samples were sent in for monitoring purposes (e.g., monitoring sera for the presence of *Mycoplasma hyopneumoniae*, PRRSV, or influenza virus antibodies, or monitoring boar semen for the presence of PRRSV).

As expected, diseases involving the gastrointestinal or respiratory systems were most common (Table 1).

Table 1. Gastrointestinal and respiratory pathology submissions

Pathology submissions	Gastro-intestinal	Respiratory system
Submissions - gross or histological	370	355
% of total swine submissions	14.1%	13.5%
Submissions of entire carcasses for necropsy	146	131
Submissions with formalinized tissue for microscopy	93	95

The importance of pathology in defining and solving enteric and respiratory problems may be related to:

- the value placed on gross and histological examination and interpretation of additional supporting lab tests, in both gastrointestinal and respiratory disease;
- many agents are involved, and they often coexist; and
- the difficulty of trying to identify and differentiate these agents either by clinical signs or gross examination alone.

Neonatal diarrheas predominated in gastrointestinal disease, with K88 enterotoxigenic *E. coli* (ETEC) being the most commonly isolated and/or identified agent. K88 ETEC was isolated in pure culture in 146 submissions, and in combination with other ETEC organisms (n = 43), from pigs ranging from 2 days to 14 weeks. Pool 2N ETEC was isolated in pure culture on 75 occasions, and in combination with other agents another 35 times, primarily from post-weaning diarrheas. Pool 1N ETEC was identified on 49 occasions in pure culture, and in mixed cultures another 16 times. Pool 1N isolates were almost exclusively from neonatal piglets.

It is unusual to examine a lung microscopically that does not have some degree of interstitial pneumonia. In most cases, a viral etiology is suspected, and occasionally subtle changes are noted that may suggest a specific etiology. However, even with additional diagnostic tests, a causative agent cannot always be identified. PRRSV infection is common, and of the total of 130 case identifications via PCR, 97 were identified in pneumonic lungs. **Gene sequencing of PRRSV is being requested with increasing frequency, and is useful in tracing the origin of new infections within production units.**

Histories accompanying submissions from which PRRSV was identified, included:

- reproductive problems, including abortions and weak neonates;
- unthriftiness, and fading nursery piglets;
- arthritis and swollen legs;
- scours and diarrhea; and most commonly,
- pneumonia, with coughing and thumping.

Other pathogens commonly identified in respiratory disease submissions (either alone or in combination with other agents) included *Streptococcus suis* (n = 133), *Pasteurella multocida* (n = 79), *Mycoplasma hyopneumoniae* (n = 41), *Haemophilus parasuis* (n = 39), *Actinobacillus pleuropneumoniae* (n = 33), porcine circovirus type 2 (n = 31), and swine influenza virus (n = 11). *AHL*

Swine influenza virus PCR is now available

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We currently offer a number of tests for the detection of swine influenza virus (SIV), including antigen detection ELISA for nasal swabs, direct FA tests, and virus isolation in embryonated eggs and cell cultures for both nasal swabs and lung tissue. **We have now added PCR for SIV for the analysis of lung tissue**, through a validation study co-sponsored by the AHL, Ontario Pork, and OMAF (see http://www.uoguelph.ca/ahl/Quality/QA%20menu.htm). The primers used identify the nucleoprotein of influenza A viruses in a single-step PCR, and can identify all strains of influenza virus that can infect swine. The fee for the SIV PCR is **$16 per test**, with testing performed at the end of each week.

For all positive PCR specimens, we strongly recommend subsequent virus isolation using both embryonated eggs and cell culture, to allow for recovery of virus isolates for multiplex PCR typing, and for ongoing surveillance of swine influenza in Ontario. As in the past, we do not charge for PCR typing from virus isolates. *AHL*

Indirect IgG immunofluorescence (IFA) test for PRRS virus

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We now offer indirect immunofluorescent antibody (IFA) testing for the evaluation of IgG antibody to the North American strains of PRRS virus in swine sera. Using the IFA test, IgG antibody can be detected in pigs as early as 7-11 days post infection, compared to 9-11 days for ELISA and 9-28 days for virus neutralization. Although IFA antibody arrives early, it persists for a relatively shorter time (4-5 months) compared to ELISA (4 to >10 months) and virus neutralizing antibody (>12 months). We are developing a similar IFA test for IgM antibody.

The IDEXX PRRSV ELISA is the best herd-based test for serological monitoring of swine herds. **The IFA test is best used to evaluate singleton PRRSV-positive reactors in PRRSV-negative swine herds.**

The IFA test is performed in 8-chamber cell culture slides at a single 1:20 serum dilution, using a virus-infected chamber and an uninfected negative control chamber for each serum. The result is reported as positive or negative. When we evaluated 25 sera from known PRRSV-positive and 25 sera from known PRRSV-negative herds, the IgG IFA had complete agreement with herd infection status.

The IFA test will be offered on Wednesdays and Fridays (on the days following routine PRRSV IDEXX ELISA testing) for a fee of **$9 per test**. *AHL*

HORSES

Ontario Racing Commission Death Registry; summary of necropsy diagnoses from 2003

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The Ontario Racing Commission (ORC) now requires that all racehorse deaths be reported if the horse has died or been euthanized within 60 days of having raced or qualified in Ontario. Once reported, the case is assessed and the requirement for a necropsy is determined. During 2003, 190 horses were registered in the ORC program, of which 125 horses were sent to the AHL for necropsy. **This program results in excellent baseline data regarding the causes of morbidity and mortality in Ontario racehorses,** providing opportunities for further research and improving equine health within this population.

Of the 125 horses necropsied, 67 (54%) were Standardbreds and 58 (46%) were Thoroughbreds. Not surprisingly, **serious fractures were the most common cause of death or euthanasia**; 63 (50%) animals were diagnosed with fractures, of which 53 (42%) were limb fractures (Table 1). The majority of fractures involved forelimbs, with only 7 of 53 (13%) limb fractures involving hindlimbs. Non-fracture related diagnoses were quite varied and involved a variety of organ systems. *AHL*

Table 1. ORC Death Registry necropsy diagnoses, 2003

Fractures 63 (50%)		**Non-fracture diagnoses** 62 (50%)			
Limb fractures	53 (42%)	Pulmonary hemorrhage	7 (6%)	Peritonitis/GI rupture	1 (0.8%)
Metacarpal 3	17	Typhlocolitis	7	Sesamoid ligament rupture	1
P1	8	Pulmonary edema/congestion	5 (4%)	Suspensory ligament rupture	1
Sesamoid	7	Undetermined cause of death	5	Suspect injection reaction	1
Carpus	6	Exercise-induced pulmonary hemorrhage	5	Laminitis	1
Metatarsal 3	4	Encephalitis/myelitis	4 (3%)	Pleuritis/pericarditis	1
Radius	2	Limb/tendon laceration	3 (2%)	Guttural pouch tympany	1
Tibia	2	Lymphosarcoma	3	Aortic rupture	1
Olecranon	2	Pneumonia	3	Hepatitis	1
Disarticulation	2	Hemoperitoneum	2 (1.6%)	Gastric ulceration	1
Humerus	1	Mesenteric artery rupture	2	Idiopathic ataxia	1
Scapula	1	Gastrointestinal accident	2	Arthritis	1
Femur	1	Colonic impaction	2		
Other - skull, rib, pelvis	10 (8%)				

Equine abortion, 2003-2004

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Although the frequency of equine abortion submissions decreased slightly this past year (Table 1), the proportion of abortions due to equine herpesvirus 1 (EHV-1) were at the highest in 10 years (data 93/94 to 97/98 not shown). **EHV-1 remains the most frequent single abortifacient pathogen identified, accounting for 18% of all abortion diagnoses.** Infectious abortion collectively comprised 35% of all abortion diagnoses, followed by non-infectious causes of abortion, primarily umbilical torsion (16%).

The submissions in 2003/2004 were received from 52 owners, however, owner identification was not given in 20 cases, making diagnoses at the herd-level problematic. Ten farms submitted 22 abortions and six of the EHV-1 abortions were received from 3 premises. Thirteen breeds were represented. Thoroughbred abortion submissions were over-represented at 48% compared to the overall AHL case submission rate of 26%, whereas Standardbred abortion submissions of 19% were lower than the overall AHL submission rate for Standardbreds of 28%. *AHL*

Table 1. Equine abortion, pathology diagnoses (number, % of total)*, AHL fiscal years 1998 - 2004

Fiscal year:	98/99	99/00	00/01	01/02	02/03	03/04
Equine herpesvirus 1 (%)	9 (14%)	8 (9%)	13 (15%)	10 (10%)	11 (12%)	15 (18%)
Infectious causes of abortion, non-viral, combined (%)	17 (27%)	25 (28%)	16 (18%)	21 (22%)	23 (25%)	14 (17%)
Placentitis	8	7	6	11	13	8
Streptococcus zooepidemicus	1	5	1	4	6	3
Miscellaneous bacteria/fungi	2	8	0	0	0	2
Mycotic	1	0	1	2	0	1
Staphylococcus aureus	0	3	0	0	0	0
Streptococcus equisimilis	2	0	0	1	2	0
Ehrlichia risticii	0	0	0	0	0	0
Klebsiella sp.	0	0	1	0	0	0
Leptospira sp.	3	0	0	1	1	0
Nocardia sp.	0	0	1	0	0	0
Lesions compatible with bacterial	0	2	6	2	1	0
Non-infectious causes of abortion, combined (%)	15 (23%)	21 (24%)	24 (27%)	26 (27%)	18 (20%)	18 (21%)
Umbilical torsion	8	12	8	15	11	13
Dystocia/stillbirth	2	5	7	6	4	3
Placental infarction	0	0	0	0	0	1
Placental adenomatous/cystic hyperplasia	0	1	0	0	2	1
Fetal goiter	0	0	1	1	1	1
Placental edema	4	3	8	0	1	0
Placental mineralization	1	0	0	4	3	0
Congenital anomalies	0	0	0	0	0	0
Idiopathic (%)	24 (38%)	38 (43%)	36 (40%)	42 (43%)	39 (42%)	37 (44%)
Total abortion cases (as % of AHL equine submissions)	64 (1.3%)	89 (1.7%)	89 (1.5%)	97 (1.5%)	92 (1.1%)	84 (0.9%)

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

Antigenic and genetic characterization of Canadian equine influenza virus isolates

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Three equine influenza-2 (AE-2) (H3N8) virus isolates recovered from Ontario horses in each of 1997, 2001 and 2002, and one AE-2 isolate recovered from a horse in Manitoba in 2001 were forwarded to the Animal Health Trust, Newmarket, United Kingdom, as part of the OIE surveillance program for equine influenza. These isolates were evaluated using cross-inhibition assays, monoclonal antibodies, and sequencing of the HA1 gene.

The 1997 Ontario isolate was typed as mainstream American lineage. The three Canadian isolates from 2001 and 2002 were typed as members of the more recent Florida/American lineage of AE-2 viruses. None of the viruses was of the European lineage.

The complete Animal Health Trust report, with details of both antibody and phylogenetic analyses can be seen on the **AHL website** at http://ahl.uoguelph.ca - click on "Newsletters, LabNotes", go to the bottom of the page and click on "Characterization of Canadian equine influenza virus isolates, 1997-2002". *AHL*

COMPANION ANIMALS

Canine leptospirosis update

Beverly McEwen, Davor Ojkic, John Prescott *bmcewen@lsd.uoguelph.ca*

Recent publications have documented a resurgence of canine leptospirosis in Canada and the USA. Increased serological submissions for *Leptospira spp*. (Table 1) reflect the concerns of our clients about this disease.

Table 1. Percent canine serological diagnoses, Ontario dogs, by year, for all *Leptospira* serovars

Year	Number submissions	Negative n (%)	Suspicious n (%)	Positive n (%)
1998	42	25 (59)	6 (14)	11 (26)
1999	54	36 (66)	10 (18)	8 (14)
2000	153	70 (45)	20 (13)	63 (41)
2001	213	101 (47)	75 (35)	37 (17)
2002	209	69 (33)	91 (43)	49 (23)
2003	**424**	**123 (29)**	**158 (37)**	**143 (34)**

There were more seropositive dogs in 2003 than in previous years, although the relative frequency was slightly lower than in 2000. The mild, wet falls of 2003 and 2000 may be associated with the relative increase of leptospirosis. **Most cases (77%) in 2003 occurred between September and December**, with fewer cases (15%) between May and August and occasional cases (8%) between January and April. Seropositive dogs were from all regions in Ontario.

The frequency of seropositivity to *L. autumnalis* and *L. bratislava* have been consistently greater than other serovars since 2000 (Table 2). Seropositivity to more than one serovar occurred in 87 of 143 (61%) positive cases, compared to 53% in 2002. Six dogs were positive to all serovars except for *L. hardjo*. **A consistent pattern of seropositivity to various serovars was not evident.** The broad seropositivity observed in these sera is probably a reflection of the involvement of several different serovars in canine leptospirosis as well as the broad cross-reactivity of IgM antibodies. IgM is the dominant immunoglobulin in the early humoral immune response.

It has still not been resolved by isolation studies whether the high frequency of *autumnalis* seropositives represents genuine infection with this serovar or is the result of cross-reacting antibodies. Interpretation of titers is also affected by immunization; dogs immunized with serovars *canicola* and *icterohaemorrhagiae* may show titers up to 320 or 640 in the first months after immunization (when of course they would be protected against these serovars). Similar relatively low serological responses are also likely to occur in dogs immunized with the newer vaccines containing serovars *L. grippotyphosa* and *L. pomona*.

Although not as dramatic as the surge of canine leptospirosis in 2000, **2003 data show that canine leptospirosis caused by several serovars has become established throughout Ontario in the last few years and, perhaps because of increased awareness, has become a more common diagnosis**. *AHL*

References

Prescott J, et al. Resurgence of leptospirosis in dogs in Ontario: recent findings. Can Vet J 2002;43:955-961.

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Table 2. Seropositivity of canine sera to various *Leptospira spp*. serovars, 1998 – 2003

Serovar	1998	1999	2000	2001	2002	2003	
	%	%	%	%	%	%	range*
L. autumnalis	4.8	3.7	30.7	11.7	20.1	28.3	320-20,480
L. bratislava	16.7	7.4	24.8	10.8	12.0	17.2	320-20,480
L. pomona	16.7	3.7	26.1	6.1	4.8	14.2	320-20,480
L. grippotyphosa	14.3	1.9	15.0	9.4	6.7	13.9	320-20,480
L. canicola	0	1.9	0	1.4	1.9	13.0	320-1,280
L. icterohaemorrhagiae	0	5.6	13.7	3.8	5.3	10.1	320-10,240
L. hardjo	-**	-	-	-	-	0.7	320

* values are the reciprocal of the titer
** serology not done

Diagnostic approach to suspected poisoning cases

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Toxicology comprises a very small portion of the caseload of most veterinary practices. Despite that, poisoning cases require special effort by everyone involved, as there is often much publicity and public emotion. This can be demonstrated by recent cases with **carbofuran toxicosis** of 16 dogs in Withrow Park in Toronto and **strychnine toxicosis** in 3 dogs from Goderich.

We were able to help the veterinarians in these recent cases because they followed a systematic diagnostic approach and sent us the proper samples.

- In the **carbofuran case**, we were able to test the source material (Figure 1) using infrared microscopy and cholinesterase levels for screening with confirmation using HPLC, GC/MS and LC/MS, with the assistance of other scientists in the Laboratory Services Division. A thorough necropsy by a pathologist and histopathology on a variety of tissues provided a direction for analytical testing.
- In the **strychnine case**, the referring veterinarian did necropsies and provided complete histories, including the environmental surroundings and the time course of events. The AHL Toxicology section was able to detect strychnine in the stomach contents by TLC.

Besides these extraordinary cases, there were 62 necropsy cases of confirmed toxicosis in dogs since 1998, out of the 101 cases of suspected poisoning submitted to us. **Ethylene glycol was the most common toxin** (n = 14), with occasional poisoning by **organophosphates** (n = 5), **strychnine** (n = 2) and **paraquat** (n = 2). Other toxins identified included dicumarol analogues, aflatoxin, roquefortine, therapeutic drugs, vitamin D, mushrooms, other plants, and ionophores. Fifty-four percent of toxicities occurred in dogs older than 5 years, 34% in dogs 1 to 5 years of age, and the rest in juveniles less than 1 year. Mixed breed dogs were most often affected (35%), followed by Labrador retrievers (8%), Siberian huskies (5%), and shelties, Golden retrievers and Cocker spaniels (3% each); a variety of both small and large breed dogs comprised the rest.

In both high profile and sporadic cases of suspected poisoning, the public looks to our profession to provide guidance in the handling and diagnosis of these cases. **Accurate diagnosis is central to provide treatment for affected animals and to prevent new cases.** Arriving at a diagnosis depends heavily on the diagnostic procedure, sample collection and handling. The approach to these cases involves more than simply requesting testing for "toxicants" in order to be successful. Such cases require a systematic approach including an extensive case history, clinical and clinical pathologic alterations, and a thorough necropsy with proper sampling. Once all the information is available, consulting with a veterinary toxicologist and pathologist about possible rule-outs will maximize the usefulness and minimize the cost of a toxicologic investigation. *AHL*

Arriving at a diagnosis depends heavily on the diagnostic procedure, sample collection and handling

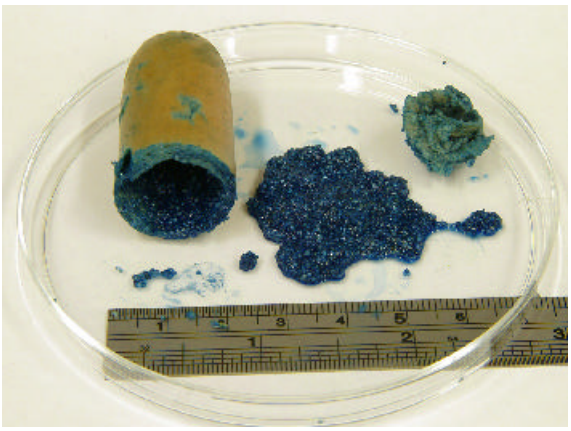

Figure 1. Hollowed-out wiener containing carbofuran granules.