UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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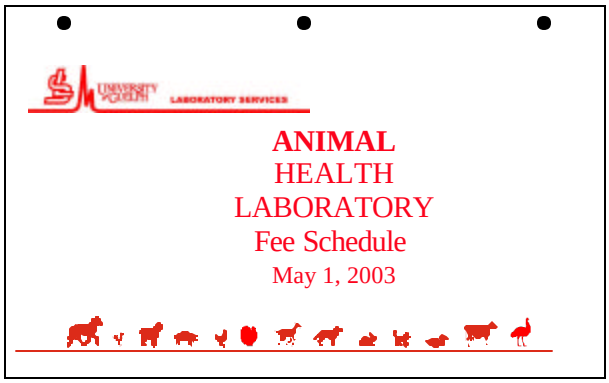
In this issue:

The May 1, 2003, AHL Fee Schedule is here

All clients should have received their new AHL Fee Schedule in late April. The enclosed cover letter explains the major changes for this edition - new tests, discontinued tests, fee changes. We've redesigned the format (alphabetical listings, Sampling/ comments, Usual day(s) tested, Turnaround time) to save you time and assist you in accessing our services. We welcome your comments.



We have a number of other aids to help you find the right test. In hardcopy and on the Web at http://ahl.uoguelph.ca are:

- **AHL User's Guide**
- ***AHL LabNotes***
  1. Update on BVD testing
  2. Tips for practitioners for field necropsies
  3. Instructions on collecting poultry blood
  4. **Nutritional and metabolic profile testing of dairy cows**

New!

Also, in this issue of the Newsletter, please see the articles on 'Which test for an infectious agent? When?', 'New molecular biology tests', and 'West Nile virus testing'. We offer several tests for a number of different infectious agents, and if can be a challenge to select the correct test to solve the problem at hand .

We welcome your feedback on all of these initiatives.

OVC redevelopment plans take shape

As part of the infusion of $113 million in federal infrastructure funds into the 4 Canadian veterinary colleges, the Ontario Veterinary College will be able to invest $37 million in the upgrading of research, diagnostic laboratory, and hospital facilities. This federal investment is being augmented by various other sources of funding, including the University of Guelph capital campaign. We are excited at the prospect of developing new and improved AHL laboratory space and processes to better serve our clients into the future.

Immunohistochemistry at the AHL

Josepha DeLay, Murray Hazlett *jdelay@lsd.uoguelph.ca*

Immunohistochemistry (IHC) tests currently available at the AHL are listed in the table below. **New tests will be posted on our website http://ahl.uoguelph.ca in the Fee Schedule and in the AHL Newsletter as they become available.** IHC tests are also used in this laboratory in surveillance programs for bovine spongiform encephalopathy (BSE), cervid chronic wasting disease (CWD), and scrapie. Tests currently under development include PRRSV, bovine herpesvirus-1 (IBR), influenza A virus, and cytokeratins. These should be on-line by June, 2003.

IHC is useful for detection of **infectious agents** in formalin-fixed tissues, particularly when fresh tissues are not available for use in other testing procedures. IHC for **cell markers** is helpful in determining the histogenesis of poorly differentiated neoplasms, and can provide important prognostic information. In most cases, the recommendation to proceed with IHC on an individual case will be based on histologic lesions, and the pathologist will communicate this information to the referring veterinarian. These tests are currently run on Tuesdays and Thursdays.

For additional information on IHC tests, please contact Dr. Josepha DeLay at (519) 824-4120 ext. 54576.

Fees for IHC are as follows:

Food-producing animals: first slide $20, each additional slide $15.

Non-food producing animals: first slide $44, each additional slide $21.

Multiple tumor markers on a single slide: first antibody $44, each additional antibody $21.

CWD/scrapie: $60 / single animal submission; $55 each for 2 or more animals. For submission information for CWD, contact Dr. Murray Hazlett at (519) 824-4120 ext. 54525. Note that specimen requirements vary with species and are being reviewed. Additional charges apply if specimen harvesting is also required. *AHL*

AHL immunohistochemistry tests - May, 2003

Infectious agents*	**Cell markers**
Transmissible gastroenteritis virus	CD3
Bovine viral diarrhea virus	CD79a
Bovine coronavirus	CD18
Porcine circovirus-2	vimentin
Toxoplasma gondii	S100
Leptospira spp	melan-A
West Nile virus	

* surveillance testing available for CWD, scrapie, and BSE

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Editorial Assistant: **Ms. Helen Oliver**

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Mailing address & contact information:
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; Fax: (519) 821-8072
Email: *holiver@lsd.uoguelph.ca*
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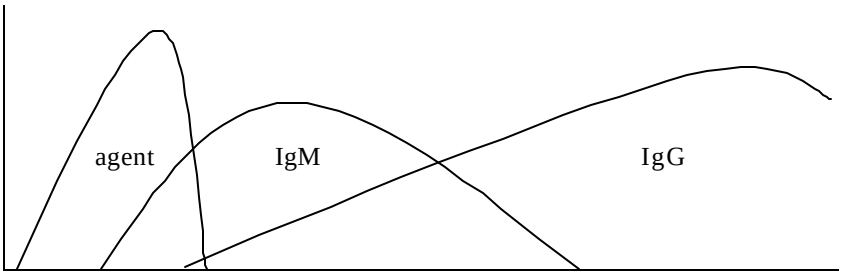
Which test for an infectious agent? When?

Grant Maxie *gmaxie@lsd.uoguelph.ca*

We offer an array of tests for detecting infectious agents - either the agent itself (**agent/antigen detection**), or the body's reaction to the agent (**antibody detection**). While this spectrum of tests is designed to uncover the various facets of an infectious disease, it can at times be bewildering. We hope that, in concert with the AHL User's Guide, the new AHL Fee Schedule will be of assistance in finding the right test and submitting the right sample. Below is a graphical overview of the usual course of an infectious disease, which gives some insight into test selection:



Time post-infection:	days	weeks-months	months-years
Examples of agents and possible test selection:	*optimal detection of ag*	*optimal detection of antibody*	
Bovine viral diarrhea virus	virus isolation (acute infection, days) virus isolation, ag detection ELISA (for PI animals) RT-PCR, IHC	virus neutralization (paired sera)	
West Nile virus	RT-PCR, IHC	equine IgM ELISA virus neutralization (paired sera)	equine IgG ELISA (surveys)
Infectious bronchitis virus	virus isolation, RT-PCR	hemagglutination inhibition IgG ELISA, AGID	
Mycobacterium paratuberculosis (Johne's disease)		IgG ELISA fecal culture/PCR (long-term, chronic infection)	
Mycoplasma hyopneumoniae	FA, IHC, PCR	IgG ELISA	

ag = antigen, ELISA = enzyme-linked immunosorbent assay, FA= fluorescent antibody, IHC = immunohistochemistry, PI = persistently infected, RT-PCR = reverse transcriptase-polymerase chain reaction

New molecular biology tests offered by the AHL

Hugh Cai *hcai@lsd.uoguelph.ca*

The following new molecular biology tests are available at the AHL and are listed in the new AHL Fee Schedule:

1. *Lawsonia intracellularis* PCR (feces or intestinal mucosa)
2. *Mycoplasma hyopneumoniae* PCR (lung)
3. *Salmonella enterica* serotype Typhimurium DT104 PCR (pure culture)
4. 16S rRNA gene sequence analysis for bacterial identification (pure culture)
5. Scrapie-resistance PrP genotyping (EDTA blood)
6. *Brachyspira hyodysenteriae* real-time PCR (feces)

(continued on page 16)

New molecular biology tests —cont'd from p 15

Lawsonia intracellularis is the causative agent of proliferative enteropathy, also known as proliferative ileitis. The disease has two clinical manifestations in pigs, an acute hemorrhagic form often called porcine hemorrhagic enteropathy, and a more chronic proliferative form often referred to as porcine intestinal adenomatosis. *L. intracellularis* is fastidious and difficult to isolate. Therefore, pathologic changes and observation of the organism in sections of fixed tissue are the conventional criteria used in the diagnosis of *L. intracellularis* infection. Recently, PCR assays have been described for the detection of *L. intracellularis* on fecal material from live pigs. We have adapted and evaluated a published PCR assay at the AHL with the financial support of Ontario Pork and Pharmacia Animal Health. Sample to submit: **feces** from live animals, **intestinal mucosa** (ileum preferred) from dead animals. Fee: free until validated, then **$16 for food animals.**

Mycoplasma hyopneumoniae is a common cause of pneumonia in grow-finish swine. The traditional isolation method is time consuming and requires special expertise. The most accurate test available for lung is deemed to be a fluorescent antibody (FA) test, which is specific and more sensitive than culture; this test however is not 100% sensitive, meaning that there will be false negatives. With the financial support of Ontario Pork and the OMAF Healthy Futures Program, we developed and evaluated a rapid PCR method to detect *M. hyopneumoniae* from swine lung tissue. Analytical validation and a small-scale field validation of the assay were completed. At the second stage of the project, Ontario Pork is supporting the AHL to perform a larger scale field validation of the PCR assay beginning May 1, 2003. To encourage submissions, we will provide **free FA and PCR tests on lung samples** until we collect enough samples.

***Salmonella enterica* serotype Typhimurium definitive type 104 (DT104)** has been identified as a major cause of salmonellosis in people and farm animals in Britain and in people in the United States. Identification of the pathogen has been based on serotyping, phage typing and multiple drug resistance profiles, which are time consuming and difficult to perform. PCR assays have been described for rapid identification of the pathogen. With the financial support of the Ontario Egg Producers, the AHL has adapted and evaluated a published PCR assay for identification of *S.* Typhimurium DT104. Sample to submit: **pure cultures on agar plates**. If other samples are to be tested, please contact Bacteriology for bacterial isolation and request PCR on pure culture. Fee: **$16/isolate**.

Sequencing of the 16S ribosomal RNA (rRNA) gene can be used to identify slow-growing, unusual, and fastidious bacteria, and bacteria that are poorly identified by conventional methods. The 16S rRNA gene is well conserved in all organisms, and contains species-specific variable regions allowing specific species identification. The AHL has evaluated the 16S rRNA gene sequence analysis method. We found that 16S rRNA gene (~1.5 kb) sequence analysis gives precise species identification. Partial gene (first 500 bp) sequencing could be used for tentative identification if your budget is tight. Sample to submit: **pure cultures**. Fee: **Complete gene $250; partial gene $100.**

Scrapie-resistance PrP genotyping. Resistance to scrapie can be determined by analysis of prion protein (PrP) sequence at codons 136, 154 and 171. The $AA_{136}RR_{154}RR_{171}$ allele is clearly associated with resistance to scrapie, and $VV_{136}RR_{154}QQ_{171}$ is clearly associated with susceptibility. With the assistance of the CFIA, the AHL has set up a DNA sequencing method for PrP genotyping. Sample to submit: **5 mL EDTA blood**. Fee**: $60 for three codons**.

Brachyspira hyodysenteriae* real-time PCR: The spirochete *B. hyodysenteriae* is the etiological agent of swine dysentery, a severe mucohemorrhagic diarrheal disease of pigs. *B. pilosicoli* is the causative agent of swine intestinal spirochetosis, a less severe, more chronic form of diarrhea in pigs and other animals. At present, the only method for diagnosis of *Brachyspira* infection in Ontario is through the microscopic appearance of the spirochetes on colonic epithelium. With the financial support of Ontario Pork and federal/provincial R & D funds, the AHL has developed a real-time PCR assay to detect *B. hyodysenteriae* from fecal samples. Analytical validation has shown that the assay is sensitive and specific. In order to perform field validation of the assay, we are providing this test on **fecal samples for free** for a limited time period (about one year). Real-time PCR for *B. pilosicoli* is under development.

For further information, please contact the AHL Bacteriology Lab or the Molecular Biology Lab.

The following personnel are involved in one or more projects in developing the above assays:

Researchers: Drs. Marie Archambault, Hugh Cai, Gaylan Josephson, Grant Maxie, Beverly McEwen, Joseph Odumeru, John Prescott (OVC), Tony van Dreumel.

Technologists: Patricia Bell-Rogers, Geoff Hornby, Carlos Leon-Velarde, Bhaju Tamot.

***Real-time PCR**: Real-time PCR is an important and relatively recently introduced technology that can detect PCR products during amplification. An optical device is connected to the reaction tubes to read the reaction signal during each cycle of the PCR reaction. Using real-time PCR, time to results is faster (20-30 minutes, compared to several hours for conventional PCR) because no extra analytical steps are required, the risk of contamination is reduced with a close-tube system, and initial template quantitation is possible by detecting the time in which the log phase of amplification appears during cycling, which is useful for determination of viral or bacterial load in a sample. (In regular PCR, amplification of different concentrations of templates could reach log phase sooner or later, so it is not possible to quantitate accurately the initial templates by measuring the end PCR products.) *AHL*

AHL Lab Reports

CATTLE

Distribution by season of major mastitis pathogens identified at the AHL, 1999 - 2002

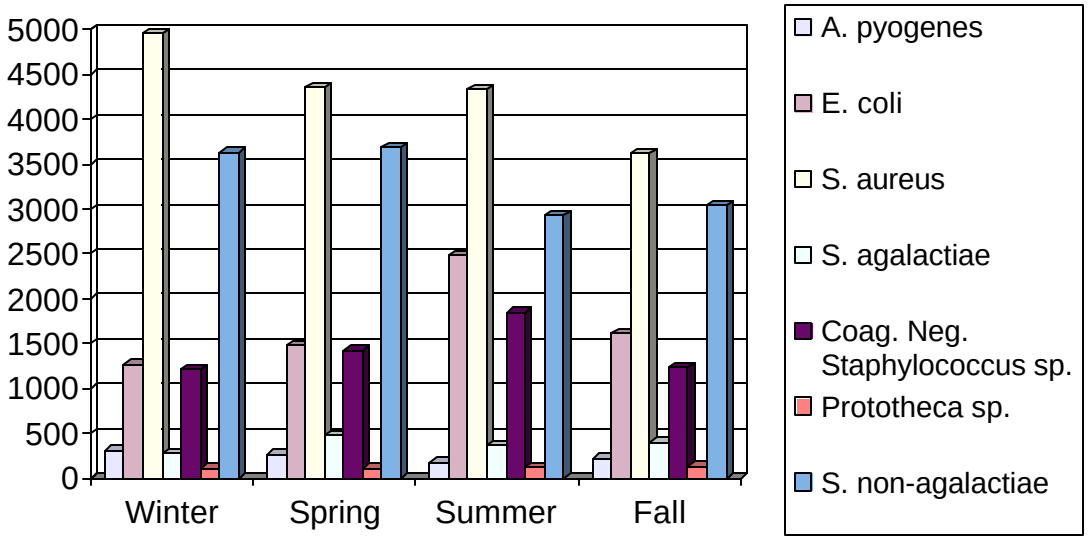
Marie Archambault, Beverly McEwen, Peter Lusis *marchamb@lsd.uoguelph.ca*

Can a season influence the spread of a particular organism? Can it be a risk factor for some pathogens, particularly those that are spread by flies or those considered waterborne organisms? **Knowledge of seasonal profiles of mastitis pathogens may be of use to practitioners in their control program selection**. Monitoring infectious organisms by season may be helpful in identifying a particular seasonal bacterial profile, as well as providing a benchmark against which to measure changes in patterns over time.

The distribution by season of *Staphylococcus aureus*, coagulase-negative *Staphylococcus* sp, *Streptococcus agalactiae*, *Arcanobacterium pyogenes*, *Streptococcus* non-*agalactiae*, *Escherichia coli*, and *Prototheca* sp identified at the AHL during 1999-2002 was evaluated. Among observations to note are:

- *S. aureus*, *Streptococcus* non-*agalactiae*, and *E. coli* were the most common mastitis-causing bacteria isolated at the AHL. *S. aureus* was the predominant organism. These findings have remained the same over the years - see AHL Newsletter, 1998; 2(3): 5.
- *S. aureus* and *Strep*. non-*agalactiae* were frequently and consistently recovered throughout the year.
- Large numbers of *E. coli* and coagulase-negative *Staphylococcus* sp were isolated during the summer.
- A greater number of *S. aureus* and *A. pyogenes* were isolated during the winter months.
- Large numbers of *S. agalactiae* isolates were recovered during the spring.
- Isolation of *Prototheca* sp was quite stable over the seasons. *AHL*

Figure 1. Distribution by season of major mastitis pathogens identified at the AHL, 1999-2002



Milk samples are usually composite samples for herd screening, or quarter samples from cows with clinical mastitis.

POULTRY

Avian influenza update

Davor Ojkic, Brian Binnington, Emily Martin, Babak Sanei *dojkic@lsd.uoguelph.ca*

Influenza A viruses have a wide host range and can infect numerous species of birds and mammals, including humans. Based on the antigenic properties of their two surface proteins, hemagglutinin (H) and neuraminidase (N), **influenza A viruses are divided into 15 H and 9 N subtypes**. Shore birds, gulls and waterfowl (especially ducks) are the major reservoir of avian influenza viruses; all 15 H and 9 N subtypes have been isolated from birds. Clinical signs caused by infection with influenza A viruses vary considerably. The main factors influencing the outcome of the disease are host species, pathogenicity of the virus, age, secondary infections, and environment. In birds, infections with avian influenza (AI) viruses are divided into two clinical forms: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). HPAI produces a severe, extremely infectious disease that is often associated with very high mortality in domestic poultry. **To date, H5 or H7 subtypes have caused all documented outbreaks of HPAI**.

> LPAI viruses most often cause mild infections, but H5 and H7 subtypes are important because they have a potential to mutate into HPAI viruses.

The latest HPAI outbreak in Ontario was in turkey breeders in 1966, and was caused by an H5N9 virus. Economic damage caused by HPAI includes both direct and indirect losses produced by the disease itself, the cost of outbreak control, and trade restrictions. LPAI viruses most often cause mild infections, but H5 and H7 subtypes are important because they have a potential to mutate into HPAI viruses. Although human disease caused by AI viruses is rare, AI also has a public health importance, because some of the H5, H7 and H9 AI viruses have zoonotic potential.

Since it may be difficult, if not impossible, to diagnose early AIV infections based solely on clinical signs, the AHL offers a battery of tests to help make a preliminary diagnosis of AIV infection:

(1) **Serological tests** available at the AHL detect the presence of antibodies against AIV in serum samples. These tests include **agar gel immunodiffusion (AGID)** and **enzyme-linked immunosorbent assay (ELISA)**. The advantage of AGID is that it can be used for any bird species, but it is important to recognize that its sensitivity varies from species to species. On the other hand, the more sensitive ELISA was designed for chickens and turkeys, but so far has been fully validated only in chickens. A positive AGID/ELISA result indicates exposure to the virus, but it is not possible to determine when exposure occurred. Early in infection, birds may be infected but still be serologically negative because it takes approximately 1 week for detectable levels of antibodies to develop. In some cases, antibodies against AI virus can be detected for a year or more after exposure.

(2) **Virus detection tests** available at the AHL include:

a) **Virus isolation (VI)** in specific pathogen-free (SPF) embryonated eggs. Virus isolation is the gold standard for virus detection; it is very sensitive and specific, but laborious and requires several days to generate results. Moreover, availability of SPF eggs needed for VI may limit our capacity to perform the test in outbreak situations.

b) **Real-time RT-PCR (RRT-PCR)** is a rapid, high-throughput test with high sensitivity and specificity. Depending on workload, results could be produced within 1 d.

c) **Antigen-capture (AC) ELISA** is a very rapid (15 min), human influenza A antigen detection kit that can be used even in the field, since it does not require specialized equipment. It is important to remember that the sensitivity and specificity of AC-ELISA are lower than VI or RRT-PCR.

Virus detection tests are most useful for virus detection early in infection. Later in the course of the disease, the virus may be cleared and birds may be negative by virus detection tests but positive serologically.

Note that HPAI is a reportable disease. The AHL is required to immediately notify the district CFIA office of any AI-positive serum samples or isolated AI viruses. **Since AIV vaccination is not practiced in Ontario, any positive AIV result is indicative of a field challenge.** If deemed necessary by CFIA veterinarians, sample(s) may be sent to the CFIA laboratory in Winnipeg for confirmation and typing. If a virus has been isolated, pathotyping and pathogenicity testing will also be carried out to determine whether or not the isolate is HPAI. *AHL*

Avian cases with viral involvement in 2002

The numbers presented in the table below are based on the cases submitted to the AHL Avian Virology lab during 2002, and include viruses from poultry, wild and exotic birds. The majority of samples originated in Ontario, but some were from other provinces.

Virus isolate	Number of cases
Adenovirus	30
Infectious bronchitis virus	27
Infectious bursal disease virus	19
Reovirus	16
West Nile virus	7
Avian paramyxovirus 1	4
Pigeon paramyxovirus	3
Turkey coronavirus	3
Canarypox virus	2
Infectious laryngotracheitis virus	1
Influenza virus	1

SWINE

Porcine respiratory disease submissions in 2002

Gaylan Josephson, Marie Archambault, Susy Carman, Tony van Dreumel *gjosephs@lsd.uoguelph.ca*

The majority of respiratory problems in swine, particularly in grow-finish animals, are due to a combination of disease pathogens, hence the name **porcine respiratory disease complex (PRDC).** Organisms involved include porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), *Mycoplasma hyopneumoniae (M. hyo)*, porcine circovirus type 2, *Pasteurella multocida, Streptococcus suis, Actinobacillus pleuropneumoniae, Actinobacillus suis, Haemophilus parasuis,* and *Bordetella bronchiseptica*.

Effective management of PRDC depends on several important steps, including:

1) identifying the pathogens involved (and these vary from farm to farm),
2) prioritizing the importance of each pathogen - a step best answered by the veterinary practitioner, who is familiar with the specific production system,
3) identifying when the agent infects the herd, using cross-sectional serological profiles, and
4) instituting effective interventions.

Step 1 is paramount, and involves postmortem examination of animals representative of the herd problem, with submission of appropriate specimens to a diagnostic laboratory. Improper samples will not provide the basis for accurate evaluation of the disease processes that are occurring, nor will they allow accurate isolation and/or culture of the pathogenic organisms. For correct sampling, refer to the AHL's new May 1, 2003 Fee Schedule, and the AHL User's Guide (both available at http://ahl.uoguelph.ca).

In 2002, 320 of the total of 2,488 swine submissions were related to disease of the respiratory tract. This does not include submissions of serum that may have been submitted for profiling purposes. The results reported here are from these submissions. No attempt has been made at prioritizing these agents.

Not surprisingly, **PRRSV was the agent most frequently involved**, being identified in 50% of the samples tested (91/180) by virus isolation, fluorescent antibody (FA), or PCR techniques, the latter using primers directed towards ORF7. *Mycoplasma hyopneumoniae* was identified in 34% (32/93) of the samples tested, using FA, PCR, and occasionally IHC techniques. Swine influenza virus was identified in 17% (13/78) of submissions, with 12 isolates being H1N1 virus and one being an H3N2 isolate. Table 1 lists the other agents identified in the submissions that were positive for PRRSV, *M. hyopneumoniae*, or SIV.

These results further confirm that: 1) porcine pneumonias are multifactorial in etiology, 2) PRRSV is the major player in swine respiratory disease in Ontario, and 3) porcine circovirus type 2 is widespread in the Ontario swine population. PRDC occurs due to the interaction and synergy of viral and bacterial pathogens, often exacerbated by environmental or management concerns.

Cost-effective management of PRDC begins with a proper diagnosis. Only then can the producer, along with his veterinarian, implement intervention strategies aimed at minimizing losses or eliminating the pathogen from the herd.

AHL

Table 1. Additional pathogens identified in submissions from which PRRSV, *M. hyo* and SIV were identified.

	PRRSV-positive (n=91)			***M. hyo*-positive** (n=32)			**SIV-positive** (n=13)		
Other agents identified	No. positive	No. tested	*% positive*	No. positive	No. tested	*% positive*	No. positive	No. tested	*% positive*
PRRS virus	-	-	-	7	12	*58*	7	12	*58*
Mycoplasma hyopneumoniae	9	42	*22*	-	-	-	2	12	*17*
Swine influenza virus	7	42	*17*	2	12	*17*	-	-	-
Porcine circovirus type 2	19	26	*73*	2	9	*22*	3	4	*75*
Pasteurella multocida	28	73	*38*	17	32	*53*	3	12	*25*
Streptococcus suis	22	73	*30*	14	32	*44*	5	12	*42*
Haemophilus parasuis	14	73	*19*	1	32	*3*	4	12	*33*
Bordetella bronchiseptica	13	73	*18*	2	32	*6*	2	12	*16*
Actinobacillus pleuropneumoniae	3	73	*4*	0	32	*0*	1	12	*8*
Actinobacillus suis	2	73	*3*	1	32	*3*	0	12	*0*

HORSES

West Nile virus testing at the Animal Health Laboratory, 2003

Susy Carman, Josepha DeLay, Davor Ojkic *scarman@lsd.uoguelph.ca*

West Nile virus (WNV) is now endemic in Ontario. The clinical signs of WNV infection in horses can include: acute onset of neurological signs (90.5%) with ataxia (85%), weakness of limbs (48%), recumbency with difficulty rising (45%), muscle fasciculations (40%), fever (23%), drooping lip (18%), twitching face or muzzle (13%), and occasionally teeth grinding (7%) or blindness (5%). Vaccination for WNV is recommended for all horses. **Because serology is the most reliable means of diagnosing WNV infection in horses, serum samples should always be taken from clinically ill suspects**. Animals can be both IgM- and IgG-positive for several weeks.

The following tests are available at the AHL for the diagnosis of WNV infection:

1. **Equine IgM capture ELISA for sera and CSF** - In horses, IgM antibodies become detectable 8-10 days post-infection and persist for 2 to 3 months. Most, but not all, horses with acute neurological disease attributable to WNV will be IgM-positive in sera. Therefore, **the equine-specific IgM ELISA is one of the most important diagnostic tests for horses.** Our fees are $30/single test, $15 each for 2-9 tests, $10 each if 10 or more. Turnaround time (TAT) = 3-5 d.

> Because serology is the most reliable means of diagnosing WNV infection in horses, serum samples should always be taken from clinically ill suspects

2. **Equine IgG capture ELISA for single horse sera** - This equine-specific test is best used for serological surveys of horse populations, and is NOT recommended for testing of individual, clinically affected horses. Our fees are $30/single test, $15 each for 2-9 tests, $10 each if 10 or more. TAT= 9-16 d.

3. **Real-time PCR** - This test can be used on any animal species. For horses presented for necropsy, fresh or frozen *brainstem and spinal cord* are the preferred specimens for PCR. *Lower regions of the spinal cord may be more heavily infected than the cerebrum when neurological signs are prominent and the animal is dying*. Because WNV antigen is sparse in horse brain, false-negative results are possible on tissues from animals with history, clinical signs, and histologic lesions in the CNS that are compatible with WNV encephalomyelitis. While WNV-specific IgM antibodies can be detected in equine CSF during clinical illness, **CSF samples do not have diagnostic value for PCR for detecting WNV RNA in horses**. Similarly, since the viremic phase of infection occurs in horses on days 3 to 7 post-infection, prior to the onset of clinical signs, equine blood-derived samples are not reliable for determining the presence of WNV by PCR. The transient low level of viremia in horses is also not considered to be sufficient to infect mosquitoes. Our fee for the WNV PCR is $34/test. TAT= 2-5 d.

4. **Histopathology and immunohistochemistry** will continue to be offered on formalin-fixed tissues. These tests can by used on any animal species. Submission of one-half of a formalin-fixed, sagittally sectioned brain (cerebrum, cerebellum, brainstem) and multiple sections of thoracic and lumbar spinal cord (if available) are recommended. Due to the scarcity of viral antigen in equine CNS tissue, **staining of MULTIPLE tissue sections having histologic lesions is recommended to reduce the possibility of false-negative results.** Our fees are $44 for the first slide, and $21 for each additional slide. TAT= 3-10 d.

Virus neutralization using paired sera - For horses and for other animal species that survive, paired sera (taken 3 weeks apart) can be tested using a virus neutralization assay to demonstrate a 4-fold rise in IgG antibody titer. Paired sera are required since IgG antibodies can persist for several years. Sera are forwarded to Cornell University for testing in a Level 3 containment laboratory. Fees are Cdn $45 per test plus $36 shipping/handling. TAT = 10 d.

The vast majority of equine neurologic cases submitted to the AHL for necropsy are treated as rabies/WNV suspects. Carcasses are held until negative rabies FA results are received, and WNV has been excluded by a combination of serology, histology and ancillary tests (IgM ELISA, PCR and/or IHC). Cases with histologic lesions consistent with WNV encephalomyelitis, but with negative IgM ELISA, PCR/IHC results are considered WNV-positive and are incinerated. As well, herpesvirus myeloencephalitis cases are treated as WNV/rabies suspects, as the clinical course is often difficult to distinguish from these other disease entities. For rare cases with long-term ataxia and a slowly or non-progressive clinical course (i.e., corresponding to typical wobbler cases), WNV and rabies are ruled out on the basis of history and lack of corresponding clinical signs such as fever.

For more information on testing for WNV, contact Dr. Susy Carman (519) 824-4120 ext 54551 scarman@lsd.uoguelph.ca, Dr. Josepha DeLay ext 54576 jdelay@lsd.uoguelph.ca, or Dr. Davor Ojkic ext 54524 dojkic@lsd.uoguelph.ca *AHL*

Highlights of AHL eastern Ontario equine necropsy diagnoses, January 2001- March 2003

Jan Shapiro, Beverly McEwen, Phil Watson *jshapiro@kemptvillec.uoguelph.ca*

Between January 2001 and March 2003, 89 horses and foals were submitted for necropsy to AHL-Kemptville from eastern Ontario, including 33 abortion/stillbirth/dead neonates (<48 h old); 14 foals at 3 d to 6 mo of age, and 42 horses older than 6 mo. The following disease highlights were selected from a review of the above cases.

Abortions, stillbirths, neonatal deaths:

- 23 of the 33 cases had gross and or histologic lesions in the fetus and/or placental tissues. In more than half of these cases, lesions were associated with an infectious agent, usually bacterial. ***Streptococcus zooepidemicus*** was the most frequently isolated bacterium. **Equine herpesvirus 1 (abortion virus)** was the next most frequently isolated infectious agents associated with abortions. A Quarter Horse foal that only lived for 1 day was diagnosed with bacterial septicemia. However, numerous **protozoal cysts** were seen in histologic sections of lung, liver and brain from the foal. Bradyzoites in some cysts stained weakly with *Neospora caninum* antigen, but did not stain with *Sarcocystis* or *Toxoplasma gondii* antigens in the immunohistochemistry tests performed by Prairie Diagnostic Services; due to the weak staining reaction, the identity of the protozoa could not be confirmed in this case.
- 11 cases had fetal and/or placental lesions of a non-infectious nature. The lesions included placental abnormalities, fetal malnutrition, asphyxia or birth trauma, hypothyroidism, and congenital and umbilical cord anomalies. **Congenital thyroid hyperplasia** was diagnosed in 1 neonatal foal that could not stand, and in a stillborn foal. The neonatal foal also had abnormal contracted forelegs and resembled cases reported in western Canada of congenital thyroid hyperplasia with multiple skeletal deformities.
- 10 cases were not associated with any fetal or placental lesions, and no infectious cause was demonstrated.

Equine submissions > 3 days old:

In the group of 56 horses older than 3 d of age, 74 diagnoses included 34 gastrointestinal, 9 nervous system, 7 musculoskeletal, 6 respiratory, 5 cardiovascular, 4 systemic disease, 2 urogenital/mammary, 1 endocrine, and 6 no specific diagnosis.

- 6 of the 11 diagnoses of **enteritis or colitis** involved *Clostridium* spp, *Salmonella* spp, or dysbacteriosis; the remaining 5 cases were diagnosed as idiopathic colitis. There was a single case in a foal of severe **ascariasis** with perforation of the small intestine, and a single foal case of **larval cyathostomiasis**. Multiple etiologic agents including **rotavirus** were implicated in 2 fatal cases of foal diarrhea. **Congenital stenosis of the distal esophagus** was diagnosed in an 8-day-old Quarter Horse foal, which had a history of having milk running out of the nostrils since it first nursed.
- A local horse with compatible neurological clinical signs and seroconversion (no necropsy) was diagnosed with **West Nile virus encephalitis**, confirming the presence of that agent in local mosquito populations.
- **Botulism** was diagnosed in a herd of 4 Arabian horses in which there was 100% mortality in a 72-hour period. The horses were fed dry hay and baylage, both of which have been implicated as sources of botulinum toxin in equine outbreaks. The source of toxin was not confirmed in this case.
- **Equine polysaccharide storage myopathy (EPSSM)** was diagnosed in a 5-year-old Belgian mare that died a few hours after becoming cast under her manger. Although she was removed from the stall, she could not stand. The cause of death was a diaphragmatic hernia presumed to have occurred during struggling as the mare tried to extricate herself. However, examination of skeletal muscle showed lesions of polysaccharide storage myopathy, which was interpreted as the likely reason the mare became cast and did not recover after she was freed. While EPSSM has been reported in many breeds of horses, a high incidence has been reported in draft breeds, and EPSSM should be considered for draft horses with a history of recumbency, including post-anesthesia, and becoming cast. *AHL*

References

1. Dubey JP, Porterfield ML. *Neospora caninum* (apicomplexa) in an aborted equine fetus. J Parasitol 1990; 76: 732-734.
2. Gray ML, et al. Visceral neosporosis in a 10-year-old horse. J Vet Diagn Invest 1996; 8: 130-133.
3. Allen AL, et al. Hyperplasia of the thyroid gland and concurrent musculoskeletal deformities in western Canadian foals: reexamination of a previously described syndrome. Can Vet J 1994; 35: 31-38.
4. Clabough DL, et al. Probable congenital esophageal stenosis in a Thoroughbred foal. J Am Vet Med Assoc 1991; 199: 483-485.
5. Lusis P, Kenney D. Botulism outbreak in horses fed baylage. AHL Newsletter 2001; 5(3): 44-45.
6. Bloom BA, et al. Postanaesthetic recumbency in a Belgian filly with polysaccharide storage myopathy. Vet Rec 1999; 144: 73-75.

Selenium toxicosis in 9 horses

Brent Hoff, Peter Lusis *bhoff@lsd.uoguelph.ca*

Selenium (Se) toxicosis was first reported in horses and cattle during the 1930's and can result from ingestion of seleniferous plants, selenium-rich soils (as in the western Great Plains of North America), or from over-supplementation with oral or injectable selenium.

Clinical signs are determined by the dose and duration of Se exposure. Relatively high doses (5 mg/kg) can cause **acute selenosis** with gastrointestinal, renal, hepatic and cardiac damage. Depression, anorexia and colic are followed by weakness, polyuria, dyspnea, and terminal shock with cyanosis and coma. **Chronic selenosis** results in hair loss and damage to epithelial structures of the hoof and skin (hair), with signs appearing 30 to 90 days after introduction of the toxic diet.

Nine horses from Eastern Ontario developed sore feet with horizontal fissures just distal to the coronary band in the most severely affected animals, and hair loss from the mane and tail in some animals. With hoof growth, the fissures deepened (Figure 1) and lameness became severe in several horses. After several weeks, all but one horse had improved clinically, but blood Se levels remained elevated at 0.47-1.2 mg/L (ref. 0.17-0.25 mg/L). Blood Se levels are decreasing, but very slowly (down to 0.28-0.56 mg/L after 3 months) and lameness remains severe in the horse with the highest levels. Analysis of the mineral mix that the horses had been given indicated 2500 mg Se/kg. Adequate levels in equine diets range from 0.30 to 2.00 mg Se/kg (ppm).

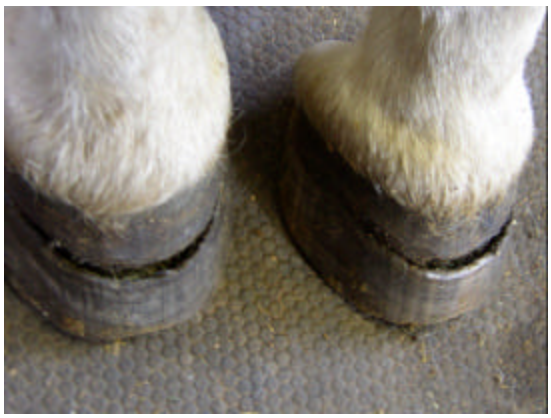


Figure 1. Hoof wall fissures resulting from selenium toxicity in a horse.

Chronic selenosis in horses can be treated successfully if owners are willing to commit to a prolonged course of nursing care. According to Dr. M. Raisbeck of Laramie, Wyoming, 6-12 months may be required to return affected animals to soundness. *AHL*

Tyzzer's disease in a 3-week-old foal

Josepha DeLay, Murray Hazlett, Art Rotenberg *jdelay@lsd.uoguelph.ca*

A 3-week-old Thoroughbred filly was found dead without obvious premonitory signs of illness. The nursing filly and her dam were housed inside at night and had access to pasture during the day, and the foal had been healthy when observed 10 hours prior to death. At necropsy, sclerae were diffusely yellow and oral mucous membranes had faint yellow discoloration. Consistent with evidence of jaundice, gross lesions were most prominent in liver and were typical of necrotizing hepatitis, with hepatomegaly and multifocal pinpoint to 2-mm white foci throughout the parenchyma.

Differential diagnoses for acute multifocal hepatic necrosis in young foals include equine herpesvirus (EHV) hepatitis (although this is most common in neonatal foals), *Actinobacillus equuli* septicemia, listeriosis, Tyzzer's disease, salmonellosis, and other bacterial septicemias. Histologically, **multifocal coalescing areas of hepatocellular necrosis were surrounded by zones of degenerate hepatocytes containing intracytoplasmic clusters of long argentophilic bacilli, consistent with *Clostridium piliforme*** (formerly *Bacillus piliformis*). Immunofluorescence for EHV 1/4 was negative on frozen sections from lung and thymus, and no bacterial pathogens were cultured from liver using either routine culture techniques or *Listeria spp.* enrichment methods.

Sudden, unexpected death is a frequent outcome of Tyzzer's disease in foals, although some infected animals will appear depressed, lethargic, or have diarrhea. Recovery has not been documented in any confirmed cases of equine Tyzzer's disease, however ante-mortem diagnosis is difficult due to inability to culture this organism using standard bacterial culture techniques. As a result, diagnosis relies on histologic demonstration of intracellular bacteria with morphology and arrangement typical of *C. piliforme* in liver.

Infection is thought to occur by ingestion of spores in soil or manure from carrier adult horses, with dissemination of spores to liver via portal circulation. ***C. piliforme* is endemic in the soil of some farms, and factors such as maternal immunity and foal stress may influence susceptibility to infection.** Although histologic examination of liver is critical for diagnosing Tyzzer's disease, samples from foals with hepatitis should also be submitted for bacterial culture, virus isolation, and immunofluorescence for EHV (liver and thymus) to rule out other bacterial causes of hepatitis/septicemia and systemic herpesvirus infection. *AHL*

References

Fosgate GT, et al. Risk factors for *Clostridium piliforme* infection in foals. J Am Vet Med Assoc 2002; 220: 785-790.

Thomson GW, et al. Tyzzer's disease in the foal: case reports and review. Can Vet J 1997; 18: 41-43.

FUR-BEARING SPECIES

Lawsonia intracellularis – A cause of proliferative enteritis in rabbits.

Emily Martin, Brian Binnington, Gaylan Josephson, Patricia Bell-Rogers *emartin@lsd.uoguelph.ca*

In the last 2 years, we have diagnosed 4 cases of proliferative enteritis in commercial meat rabbits at the Animal Health Laboratory. *Lawsonia intracellularis* was identified in all 4 cases. The presenting complaint involved ongoing diarrhea and mortality in rabbits 3-12 weeks of age. In one case, rabbits had been moved into an area previously used for pig housing.

***Lawsonia intracellularis* is a bacterium that causes primarily a proliferative enteropathy in multiple species and can cause interspecies infections**. The clinical presentation has been recognized for decades, but the etiologic agent was not identified until 1995. Infection is primarily limited to the intestinal mucosae, and there is little or no dissemination to other body systems. The intestinal location varies among species. In the rabbit, the jejunum and proximal ileum are typically affected, but lesions have also been identified in the large intestine. **The organism grows in the apical cytoplasm of enterocytes**. The bacterium is obligately intracellular, highly fastidious, requires growing cells for in vitro maintenance, and is located in a highly contaminated site. All of these factors make culture and identification difficult.

Clinical presentation of acute *Lawsonia intracellularis* infection involves diarrhea and mortality in suckling, weanling, and young-adult domestic rabbits

Clinical presentation of acute infection involves diarrhea and mortality in suckling, weanling and young-adult domestic rabbits. On post mortem, the colon and the rectum may contain semi-fluid material. In more chronic cases, the small intestine appears thickened and corrugated. Microscopically, the lesions vary from suppurative and erosive to proliferative. Silver stains demonstrate intracytoplasmic clusters of bacteria in the apical cytoplasm (Figure 1).

Diagnostic methods at the AHL are currently based on gross post mortem findings, histologic lesions, and Warthin-Starry silver stains. A PCR test for *L. intracellularis*, which is not validated in rabbits, has been developed at the AHL. All 4 cases at the AHL were diagnosed using gross and histologic evaluation. PCR test development was initiated only recently, therefore only 2 of the 4 cases with typical gross and histologic lesions were also submitted for PCR testing, and both were positive.

Due to the recent identification of the *Lawsonia intracellularis* bacterium as an etiologic agent of proliferative enteropathy, the pathogenesis of the disease is still being elucidated. Transmission is likely by ingestion of fecal matter, and co-infections with other bacteria (e.g., *E. coli*) are possible. A better understanding of the organism will aid in future disease management and treatment. *AHL*

References

1. Smith DGE, Lawson GHK. *Lawsonia intracellularis*: getting inside the pathogenesis of proliferative enteropathy. Vet Microbiol 2001; 82: 331-345.

2. Percy DH, Barthold SW. Pathology of Laboratory Rodents and Rabbits. Iowa State University Press, Ames, Iowa. 2001: 272-274.

3. Lawson GHK, Gebhart CJ. Review: Proliferative enteropathy. J Comp Pathol 2000; 122: 77-100.

4. Jordan DM, et al. 1999. Detection of *Lawsonia intracellularis* in swine using polymerase chain reaction methodology. J Vet Diagn Invest 1999; 11: 45-49.

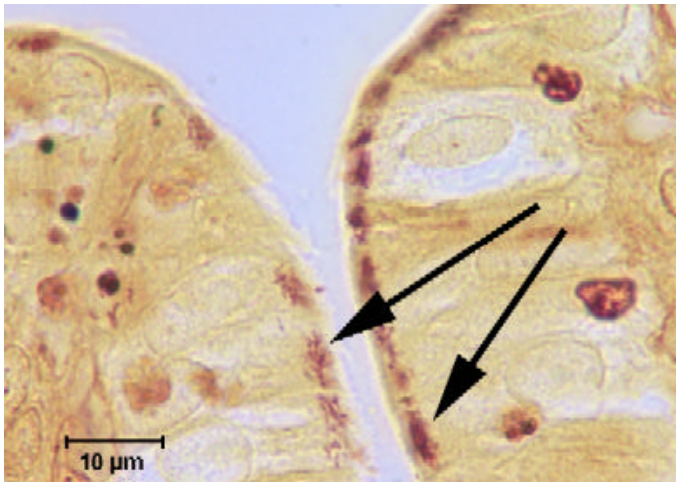


Figure 1. *Lawsonia intracellularis* (arrows) in the apical cytoplasm of intestinal mucosa. Warthin-Starry silver stain.

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)

The highest frequency of seropositive dogs (titer >320) occurred in 2000. Following a decrease in the proportion of seropositive dogs in 2001, a mild increase occurred again in 2002. Last year was unusual in that the majority of serodiagnoses were in the 'suspicious' category (titers between 80 and 160). **Although canine leptospirosis tends to follow a seasonal pattern, cases do occur throughout the year.** In 2002, as usual, most cases occurred in the fall and early winter (Sept-Dec); May was the only month when there were no positive cases. Seropositive dogs were from all regions of Ontario (eastern, central, south-central, north, and Toronto).

The frequency of seropositivity to *L. autumnalis* and *L. bratislava* have been consistently greater than other serovars since 2000 (Table 2). The range of seropositive titers varied amongst serovars with highest titers occurring to *L. autumnalis* and *L. grippotyphosa*.

Seropositivity to more than one serovar occurred in 26 of 49 positive cases. Only one dog was positive to all serovars. **A consistent pattern of seropositivity to various serovars was not evident.** 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).

Although not as dramatic as the surge of canine leptospirosis in 2000, **2002 data show that canine leptospirosis caused by several serovars has become established throughout Ontario in the last few years**. *AHL*

References

1. Prescott J, et al. Resurgence of leptospirosis in dogs in Ontario: recent findings. Can Vet J 2002; 43: 955-961.
2. Ward MP. Clustering of reported cases of leptospirosis among dogs in the United States and Canada. Prev Vet Med 2002; 56: 215-226.

Table 2. Seropositivity of canine sera to various *Leptospira spp*. serovars, 1998 – 2002

	1998	1999	2000	2001	2002	
	%	%	%	%	%	range*
L. autumnalis	4.8	3.7	30.7	11.7	20.1	320-20,480
L. bratislava	16.7	7.4	24.8	10.8	12.0	320-5,120
L. grippotyphosa	14.3	1.9	15.0	9.4	6.7	320-20,480
L. pomona	16.7	3.7	26.1	6.1	4.8	320-2,560
L. icterohaemorrhagiae	0	5.6	13.7	3.8	5.3	320-640
L. canicola	0	1.9	0	1.4	1.9	320-5,120

* values are the reciprocal of the titer

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