UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Email reports - the way to go!

Many of our clients are opting to receive reports from the AHL computer system (VADDS) by email rather than fax. Email reports are sent in 'real-time', i.e., immediately, whereas faxed reports go into a queue and are sent in sequence. Faxing can fail due to machine problems - no paper, multi-use lines (phone/fax/answering machines), machine switched off - causing delays in fax transmission.

If you would like to switch your results to email transmission, please contact Ms. Mary Halfpenny, our information technology supervisor:

mhalfpen@lsd.uoguelph.ca

If you have questions about this option, please call Mary at 519-824-4120, ext 54563.

Congratulations Dr. Archambault!

We are delighted to announce that **Dr. Marie Archambault**, AHL veterinary bacteriologist, passed the rigorous American College of Veterinary Microbiology examinations in November, 2002, and is now a 'Diplomate ACVM'. Marie joined the AHL in August, 1999. A 1993 DMV graduate from La Faculté de médecine vétérinaire de l'Université de Montréal, St. Hyacinthe, she subsequently completed an MSc (1995), and PhD (1999).

Dr. Archambault oversees a diverse group of activities, including diagnostic bacteriology and mycology, mastitis herd testing, and hatchery bacteriology, and provides interpretation of bacteriology lab results. Work is also proceeding well in her lab section on validation of new tests for detection of Johne's disease, plus other developmental projects.

Submission of live animals to the AHL

- **We ask that live large animals be euthanized before transport to the AHL,** in view of the very limited live-animal handling facilities at AHL-Guelph and AHL-Kemptville, and our inability to hold live animals overnight.
- **Live calves and piglets** remain the preferred submission in cases of herd outbreaks of diarrhea or pneumonia - preferably acutely affected, untreated individuals that are representative of the herd/flock problem.
- **Please call ahead if you wish to submit live animals**. We can then notify staff of impending arrival.
- Please note that a **$75 surcharge** is applied to PM cases that arrive after 4 PM.
- Live large animals >100 kg submitted to AHL-Guelph may be referred to the Veterinary Teaching Hospital for euthanasia at an extra charge.

Transmissible spongiform encephalopathy (TSE) update

Murray Hazlett, Josepha DeLay, Aru Balachandran *mhazlett@lsd.uoguelph.ca*

When veterinarians collect formalin-fixed obex for chronic wasting disease (CWD) testing, **we ask that they also freeze some adjacent brain tissue** (just caudal or cranial to the piece selected for immunohistochemistry (IHC)). In the event of a test in which the obex is disrupted and the vagal nuclei cannot be found, further testing can be done by CFIA using a **Western blot** technique on the frozen samples. If you receive results categorized as "inappropriate sample" or "no specific staining seen", you can then send the frozen tissue to the AHL for forwarding to CFIA. This test will also be useful in cervids found dead (autolysed) without any premonitory signs, or showing nervous, wasting or respiratory signs, to rule out CWD as the cause of death. It is hoped that this will be an acceptable alternative test that will allow for herd certification in cases of autolysed animals or those where the vagal nuclei could not be located.

Since beginning immunohistochemical screening programs in 2002, we have not detected BSE or CWD in Ontario by IHC testing (Table 1).

At the TSE training course, Weybridge. From left, Drs. Murray Hazlett, Gerald Wells (discoverer of BSE), Josepha DeLay.

In late November, a TSE training course in Weybridge, England was attended by Drs. Murray Hazlett and Josepha DeLay of the AHL. The course dealt primarily with bovine spongiform encephalopathy (BSE) diagnosis, but also involved new rapid tests that could be incorporated into CWD testing regimes. The **Bio-Rad ELISA has recently been approved by the USDA for CWD testing**, and is undergoing validation by CFIA. *AHL*

Table 1. TSE immunohistochemistry tests performed by the AHL in 2002

Species/test	Bovine (BSE)	Elk/Red deer (CWD)	White-tailed Deer (CWD)
Total tests done	678	119	190
Number of CFIA[1]	9	1	9
Positive tests	0	0	0

[1]Sent to CFIA Winnipeg (BSE) or Nepean (CWD) for confirmatory negative testing.

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Zoonotic pathogens and diseases identified at the AHL, 1998 - 2002

Beverly McEwen, Marie Archambault, Davor Ojkic, Susy Carman, Josepha DeLay, Peter Lusis, Murray Hazlett *bmcewen@lsd.uoguelph.ca*

Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens: in 2002 AHL staff identified ***Mycobacterium bovis* infection in a cattle herd**, and **West Nile Virus infection in horses.** Other zoonotic pathogens isolated and/or identified at the AHL are given in Tables 1 & 2. These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates.

Salmonella sp, Streptococcus suis, Streptococcus zooepidemicus and *Cryptosporidium sp.* are the most frequently identified zoonotic pathogens at the AHL since 1998 and have increased since 2001. In cattle and swine, *S. typhimurium* is the most common serotype isolated and of these, the multiresistant phage type 104 is most frequent. Occupational exposure to pigs and horses is a risk factor for *S. suis* and *S. zooepidemicus* infections, although consumption of unpasteurized milk or dairy products from cows with *S. zooepidemicus* mastitis resulted in human infections.

As part of our commitment to health surveillance, **the AHL developed new tests in 2002** such as immunohistochemistry for bovine spongiform encephalopathy, West Nile virus, toxoplasmosis, and molecular techniques to identify *Leptospira spp.* and verotoxigenic *E. coli.* AHL

Table 1. Zoonotic pathogens isolated and/or identified at the AHL, 1998 – 2002

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	**2002**	2001	2000	1999	1998
Campylobacter coli/ jejuni/ fetus subsp. fetus				6				21		6	**33**	36	14	6	11
Chlamydophila psittaci				5	4					1	**10**	12	21	14	13
Coxiella burnetii (Q fever)					6						**6**	7	4	4	2
Cryptosporidium sp.	185	5		1	8						**199**	160	120	93	127
Francisella tularensis										1	**1**	3	6	0	0
Giardia sp.	3		1								**4**	19	26	4	14
Listeria monocytogenes	21				9					7	**37**	44	34	16	22
Methicillin-resistant *Staphylococcus aureus*			11					2			**13**	1	-	-	-
Mycobacterium bovis[1]	1										**1**	0	0	0	1
Rabies[1]											**0**	5	1	1	0
Salmonella sp[2]	160	142	121	9	1	39	33	7	3	201	**716**	565	754	711	671
Streptococcus suis	12	463			1					1	**477**	380	560	489	664
Streptococcus equisimilis		110	22					4		8	**144**	126	154	93	110
Streptococcus zooepidemicus	2	2	212					3	3		**222**	175	162	220	134
Toxoplasma sp				7	2			1	2		**12**	12	8	13	15
Verotoxigenic *E. coli*[3]											**0**	0	3	1	1
West Nile Virus			84							7	**91**				
Yersinia enterocolitica		6	1							1	**8**	5	8	2	9

[1] Federally reportable diseases confirmed by the Canadian Food Inspection Agency

[2] *Salmonella sp* serotyping and phage typing done by Laboratory for Foodborne Zoonoses, Health Canada

[3] Under-represented as VTEC confirmation requires specialized methodology done on request (see AHL Newsletter June 2001, p. 25).

Table 2. Number of *Leptospira spp*. seropositive samples (>1:160) identified at AHL, 1998 – 2002, microscopic agglutination test (MAT)

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Feline	**2002**	2001	2000	1999	1998
L. autumnalis				45	1	**46**	32	50	2	2
L. bratislava		85	29	25		**139**	120	88	116	22
L .canicola				4		**4**	6			
L. grippotyphosa		10	5	14		**29**	44	32	18	61
L. hardjo	30					**30**	21	7	16	1
L. icterohaemorrhagiae	148	2	1	11	1	**163**	289	140	224	31
L. pomona	43	38	14	10		**105**	110	112	152	40
Total	**221**	**135**	**49**	**109**	**2**	**516**	**622**	**429**	**528**	**157**

AHL Lab Reports

CATTLE

Ear notch testing for the identification of BVD virus-PI calves: which test to request, and are there better options?

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

When using ear notches for the identification of calves less than 3 months of age that are persistently infected (PI) with BVD virus, we currently recommend that ear notches be tested using the BVDV antigen detection ELISA.

The AHL uses the Syracuse Bioanalytical BVD Virus Antigen Detection ELISA, which is licensed for the detection of PI animals by the Canadian Food Inspection Agency and approved for the testing of ear notches by the USDA. Comparison studies show this ELISA to be of similar sensitivity to immunohisto-chemistry (IHC) for the identification of calves persistently infected with BVDV (1).

IHC on ear notch samples or skin biopsies to identify PI calves has been in vogue for several years. However, IHC on ear notches is not routinely offered at the AHL. The IHC test is much more expensive for clients ($65 minimum, vs. **$10 per ELISA test**). The IHC requires longer sample processing time, whereas the ELISA is a rapid test that can be completed in one day.

> We currently recommend that ear notches be tested using the BVDV antigen detection ELISA

Ear notches are collected using swine ear-notch pliers. Notches should have a minimum of 1-cm cut edge. This size can be obtained by adjusting the position of the swine ear notch pliers on the ear tip. Swine ear-notch pliers, which are ~8 mm wide at the base with ~1.5 cm sides, can be purchased at farm supply stores. To prevent transfer of disease, they must be disinfected between calves. This can be done by rotating the use of 3-4 units, with soaking in disinfectant between calves. To remove the disinfectant, be sure to rinse carefully in water and dry the pliers before use on the next calf. **Submit ear notches in individually labeled tubes without additives.** For long-term storage, samples can be frozen prior to submission to prevent dehydration.

For calves less than 3 months of age, instead of ear notches, blood can be tested using PCR to identify BVDV-infected calves (2). For animals over 3 months of age, serum or plasma can be readily used in both the ELISA and the PCR test. Blood samples are less traumatic to collect, with easier maintenance of sterility between animals.

At the AHL, the BVD Virus Antigen Detection ELISA is routinely performed **two times each week** (Wednesdays and Fridays) with results available late afternoon. If your test is urgent, please let us know as soon as possible so we can plan to test your specimens on the day they arrive. This ELISA is a very good and rapid test intended to identify persistently infected carriers, with high levels of viremia. However this ELISA should not be used alone to identify "acutely infected" animals, where the level of viremia in blood may be below the limits of detection of the ELISA (minimum virus detected is log 10^4/mL). For complete instructions on BVD testing in various clinical situations, please see the BVD LabNote on the AHL website (http://ahl.uoguelph.ca ; go to the link for Newsletters/LabNotes, then to Update on BVD Testing) or phone Dr. Susy Carman at 519-824-4120 ext 54551). *AHL*

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2. Deregt D, et al. A comparison of polymerase chain reaction with and without RNA extraction and virus isolation for detection of bovine viral diarrhea virus in young calves. J Vet Diagn Invest 2002; 14: 433-437.

Bovine respiratory syncytial virus pneumonia

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

Bovine respiratory syncytial virus (BRSV) is one of the most important pathogens causing respiratory disease in all ages of cattle that are submitted to the AHL. The proportion of bovine respiratory disease diagnoses attributed to BRSV over the last 5 years is listed in Table 1.

Table 1. BRSV as a proportion of respiratory pathology cases

Fiscal yr, May 1 to April 30	Number of diagnoses of BRSV	BRSV as a proportion of all respiratory pathology cases
1998-1999	17	5.1%
1999-2000	24	7.5%
2000-2002	9	2.7%
2001-2002	41	8.6%
May – Dec 2002	30	9.2%

Gross necropsy lesions vary from consolidation of cranioventral lung lobes to interstitial pneumonia with consolidation, interlobular edema, and emphysema involving all lung lobes. On **histopathology,** there is acute bronchointerstitial pneumonia with bronchiolitis and alveolitis. In many cases, there are multinucleated (syncytial) cells present in affected airways and alveoli. Parainfluenza type 3 (PI-3) virus can cause similar histological lesions. However clinical pneumonia due to PI-3 virus is less common in Ontario.

Because similar histologic lesions can be caused by PI-3V, the following tests need to be performed in addition to **histopathology** to establish a definitive diagnosis of BRSV pneumonia:

- **fluorescent antibody test for BRSV on three pieces of frozen lung selected from the edge of the lung lesions,**
- **serology on acute/convalescent sera,**
- **immunohistochemistry** can also be applied to formalin-fixed lung tissue when fresh or frozen tissues are not available. *AHL*

It is important to **submit both formalin-fixed and frozen lung,** as well as **paired sera,** to establish a definitive diagnosis of BRSV pneumonia.

Hypokalemia syndrome in dairy cows may resemble botulism

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

Hypokalemia in cattle may be secondary to other conditions, such as renal dysfunction, diarrhea, anorexia, or a protracted illness. **A syndrome of profound weakness and recumbency has been recognized in adult dairy cattle with marked hypokalemia**. Several reports indicate that affected animals have very low serum potassium concentrations (range 1.35 to 2.5 mmol/L, ref. 4.0 to 5.0 mmol/L). Approximately 50% of these cows had been treated for fatty liver with isoflupredisolone before onset of clinical signs.

Most affected animals show muscle weakness similar to that seen with botulism. Although this muscle weakness can be profound, only rarely was dysphagia noted with hypokalemia cases. **The reports indicate that this muscle weakness affects the neck muscles to the point were the neck "flops" to the side or swings from side to side**. This profound weakness typifies hypokalemic myopathy, but not botulism. Cows with botulism may be recumbent (resembling milk fever), and the tongue will hang out one side of the mouth, with loss of normal lingual tone. There is also a lack of prompt response to retract the tongue into the mouth after manual traction.

Profound muscular weakness typifies hypokalemic myopathy, but not botulism

AHL records contain 149 cases of profound hypokalemia (range 1.0 to 2.5 mmol/L) over the 4-year period 1998 to 2002, with the most common clinical signs noted as: recumbency, abnormal position of the neck, severe weakness, rumen hypomotility, anorexia, and tachycardia. *None of the histories of these cases indicated total loss of lingual tone, but most did indicate very weak neck muscles. Clinical feedback on the lingual tone from these cases would be appreciated.*

Response to treatment is disappointing, but some cows may recover with oral treatment with potassium administered as soon as possible after onset of clinical signs. *AHL*

References

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2. Whitlock RH, et al. Botulism toxicosis of cattle. The Bovine Proceedings. 1999: 45-51.

POULTRY

Molecular characterization of Ontario IBDV field strains

Davor Ojkic, Janet Swinton, Gaye Smith, Brian Binnington *dojkic@lsd.uoguelph.ca*

The Animal Health Laboratory has introduced new serologic and molecular diagnostic methods to enhance its capabilities in detection and characterization of field infectious bursal disease viruses (IBDV's). During this study, IBDV samples submitted to our laboratory were subjected to molecular characterization in order to determine whether "variant" IBDV's are present in Ontario. The samples originated from flocks with conditions such as gangrenous dermatitis, *E. coli* septicemia, inclusion body hepatitis, and/or a history of production problems without overt clinical signs. The ages of the flocks from which the samples were collected ranged from 14 to 48 days. Virus was identified in bursae with moderate-to-severe and acute-to-chronic bursal damage. IBDV was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in 33 bursal samples that had histopathological changes suggestive of IBDV infection. Following RT-PCR, the samples were characterized by restriction fragment length polymorphism (RFLP) analysis to place these field isolates into genetically related molecular groups.

North American IBDV strains are currently classified into six molecular groups: variant viruses and their vaccines belong to **groups 1 and 2**, classic viruses and vaccines based on these viruses are in **groups 3, 4 and 5**, while viruses in the newly established **group 6** are ambiguous (1). IBDV field strains that belong to the same group are likely antigenically related (2). Four groups of RFLP patterns were observed from the Ontario isolates:

1. 5 field strains had the RFLP pattern compatible with molecular group 2 viruses.
2. 1 field strain had the RFLP pattern compatible with molecular group 5 viruses.
3. 16 field strains had the RFLP pattern compatible with the molecular group 6 viruses.
4. 11 field strains had a "new" RFLP profile.

In order to more precisely investigate the genetic background of field IBDV strains, in particular the ones that were untypable by RFLP analysis, **partial amino acid (aa) sequences of the viral coat protein (VP2) gene from selected field strains were determined**. VP2 gene sequences from viruses with a molecular group 2 RFLP profile were 98.2-100% identical to Delaware E IBDV strain. VP2 aa sequences from IBDV's with molecular group 6 RFLP profiles showed highest identity, 95.9-98.1%, to IBDV strain 586 (3).

> These results demonstrate that variant IBD viruses, such as Delaware E, are present in Ontario

Interestingly, most VP2 gene sequences from viruses with "new" RFLP profiles were almost 100% identical to Del E, but one virus showed the best match, 97.4%, to molecular group 6 viruses. These results demonstrate that variant IBD viruses, such as Delaware E, are present in Ontario. Furthermore, **we also found that new RFLP profiles need to be examined by VP2 gene sequencing to more precisely investigate the spectrum of genetic changes that may not be detected by the RT-PCR/RFLP analysis**. *AHL*

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2. Jackwood DJ, Sommer SE. Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses. Avian Dis 1997; 41: 627-637.
3. Smiley JR, Jackwood DJ. Genetic stability of the VP2 hypervariable region of four infectious bursal disease virus isolates after serial passage in specific-pathogen-free chicken embryos. Avian Dis 2001; 45: 1-8.

Infectious bursal disease test availability update - IDEXX IBDxr ELISA

The AHL recently replaced the IDEXX IBD classic ELISA with the **IDEXX IBDxr (extended range) ELISA**. The IBDxr ELISA features enhanced detection of antibodies to variant IBD strains and has better correlation to virus neutralization compared to the classic IBD test kit. The price remains the same at $2.75/sample.

Highlights of AHL Ontario poultry pathology diagnoses, 2001 - 2002

Brian Binnington, Emily Martin, Beverly McEwen, Jan Shapiro *bbinning@lsd.uoguelph.ca*

The following disease highlights have been selected from the yearly summaries of disease entities that are produced from the AHL database of pathology diagnoses made at the Guelph and Kemptville AHL laboratories. These disease entities were identified from May 2001 to September 2002. Some of these disease conditions represent continuous problems while others may indicate changing trends that require further investigation and surveillance.

Broiler chickens

Bursal damage and atrophy with acute to chronic lymphocyte necrosis and depletion were frequent findings in birds submitted for necropsy. The bursal damage occurred most frequently at 3-4 weeks of age, after maternal antibodies had disappeared. Bursal damage was a frequent finding along with concurrent diseases such as **coccidiosis** (usually cecal), **necrotic enteritis** (***Clostridium perfringens***), systemic bacterial infections (usually ***E. coli***), or in birds that were submitted because of unevenness and poor production parameters in previous crops. Some of the bursal tissues were tested and found to be positive for **infectious bursal disease virus (IBDV)** by molecular techniques of PCR and gene sequencing. The results are presented in Dr. Davor Ojkic's article in this newsletter. No consistent thymic or bone marrow depletion suggestive of **chicken anemia virus (CAV**) infection was identified in these cases.

Inclusion body hepatitis associated with **adenovirus** was identified on gross and histopathological examination as a sporadic cause of mortality in young 1-4 week old birds. However, clusters of cases with higher morbidity and up to 30% mortality have been identified in the last 2 years, associated with a few specific flocks of breeder birds. The relationship of the adenoviruses in the affected broilers and their parent breeder flock is poorly understood. The interactions of vertical transmission, horizontal transmission, the protection by maternal antibody in the chick, the strains of viruses involved, and the pathogenicity of these viruses are not known.

The number of diagnoses of **ascites and acute death syndrome ('flip-overs')** were 3-4 times less frequent than in the previous 3 years, perhaps associated with milder winters. Respiratory diseases, especially those associated with **infectious bronchitis virus (IBV)** have declined over the previous 2 years, with only 3 cases of IBV tracheitis identified this past year. Bone deformities seen, including **valgus/varus, tibial dyschondroplasia, spondylolisthesis (kinky back), and rickets,** have been decreasing since the late 1990's.

Broiler breeder chickens

Bacterial infections continue to be the most commonly identified cause of disease, resulting in **septicemia, arthritis, tenosynovitis**, **osteomyelitis, cellulitis,** and **pododermatitis**. ***E. coli*** and ***Staphylococcus aureus*** are the primary causes of these infections in adult birds. Neoplastic diseases such as **myelocytomatosis** associated with **J-type avian leukosis virus** continued to decline over the past year. **Urate nephrosis with visceral urate deposition** caused mortalities in 5 flocks. These cases occurred with equal frequency in winter and summer months. No evidence of nephropathogenic IBV's could be identified in any of the affected birds.

Layer chickens

Nutritional and metabolic diseases continue to be frequent causes of morbidity and mortality in laying hens. **Calcium depletion** in laying hens can result in sudden death due to hypocalcemia during the egg-shelling process or, more prolonged depletion can cause **osteomalacia/cage layer fatigue** with resultant bone fragility and fractures. **Fatty liver and hemorrhage syndrome** was a problem in some flocks. Dehydration with **urate nephrosis and visceral urate deposition** has occurred more frequently in the last two years. The causes of this problem in laying hens are often multiple and not simply due to water deprivation. Trauma associated with **cannibalism** results in significant vent lacerations and hemorrhage, peck-outs and dermatitis over the back. Cannibalism occurred in both small and large operations usually in older birds of 40 to 60 weeks of age. Respiratory diseases were infrequently diagnosed this year, with tracheitis associated with **infectious bronchitis virus** identified on 2 occasions. **Infectious laryngotracheitis (ILT)** due to **herpesvirus** was occasionally seen in small backyard flocks and in large, multi-age operations. In the multi-age operations, it affected young birds prior to their vaccination for ILTV. Spread of vaccine virus from older birds that had been vaccinated recently was the likely cause of the infections. **Necrotic enteritis (*Clostridium perfringens*)** was identified more frequently this past year. There were 2 age groups affected, either pre-lay (6-12 weeks old) or in-lay birds (24-56 weeks old).

Turkeys

Bacterial infections are the most frequently identified cause of disease in turkeys. ***E. coli*** is responsible for septicemia in all age groups. Multiple serotypes of ***Salmonella sp.*** bacteria caused intestinal infections, usually without systemic disease. **Necrotic enteritis** due to ***Clostridium perfringens*** has been identified more frequently in the last 2 years; while found alone, clostridial enteritis was usually associated with **coccidiosis**. **Nutritional and metabolic bone disease (rickets)** was increased 3-4 times over the previous 3 years. Rickets was identified in both small flocks of 20 to 100 birds and larger flocks of 6-12,000 birds. The lesions in several flocks resembled **fibrous osteodystrophy**, which is a disease that has been associated with an increase of phosphorus relative to calcium in the ration of various animal species. In contrast, **long bone deformities**, such as tibial rotation, tibial dyschondroplasia and valgus/varus deformity, were only identified once this year. *AHL*

SWINE

PRRS and swine influenza in a nursery

Gaylan Josephson, Susy Carman, Tony van Dreumel, Doug MacDougald *gjosephs@lsd.uoguelph.ca*

Porcine respiratory disease often poses a diagnostic dilemma to the veterinary practitioner. Identifying which of the many pathogens are of primary significance can be difficult. Viral agents such as PRRS virus, circovirus type 2, and swine influenza virus (SIV) along with bacterial agents such as *Mycoplasma hyopneumoniae, Streptococcus suis, Haemophilus parasuis, Actinobacillus suis, Actinobacillus pleuropneumoniae*, and *Pasteurella multocida* are often identified in various combinations. It has become increasingly difficult to assess the importance of swine influenza in respiratory disease.

There are two clinical forms of swine influenza. The more readily recognized **"acute, explosive" form** occurs in immunologically naive groups of swine as an abrupt onset of clinical disease. Pigs are sick for a few days with fever, anorexia, dyspnea and a barking cough. The disease moves rapidly within the group to affect the entire facility within a few days, before it disappears. A carrier state does not occur.

In contrast, the **"endemic" form** of swine influenza occurs in herds with partial immunity or in herds with continuous addition of naive animals into the group. In this situation, swine influenza is more insidious, being found all year round, and is an important contributor to the "porcine respiratory disease complex".

> The severity of the respiratory outbreak in these nurseries was related to the combination of agents identified

In late October 2002, a "porcine respiratory disease complex" was identified in nursery piglets in a large farrow-to-finish operation. Piglets were sourced from 3 sow herds, were weaned at 14-21 days of age, and filled individual nurseries within a 10-day period. Piglets were commingled within the off-site nurseries, which operated as all-in/all-out by site. Coughing was noted in the piglets shortly after they entered the nursery, but was not considered to be a problem. Two to three weeks post entry, a severe respiratory problem occurred in piglets in barn A. Death losses increased, and average daily gains decreased in spite of aggressive antibiotic therapy. Post-mortem examination of several piglets revealed extensive cranioventral lung consolidation with overlying fibrinopurulent pleuritis. **Pathogenic agents identified from lung tissue included *Streptococcus suis, Salmonella* Typhimurium var. *copenhagen, Haemophilus parasuis,* and a field strain of PRRS virus with an RFLP cut pattern of 1-2-4.**

Pigs in the next barn to be filled (barn B) broke with a similar respiratory problem 2 weeks post-entry. Total mortality and average daily gain of pigs in this barn were similar to those of barn A. Clinical, gross post mortem and microbiological findings were also similar. **Piglets were positive for PRRS virus using both immunofluorescence and PCR**. Because of the nature of the cough (a harsh, deep bronchial cough was noted in several animals), nasal swabs were taken from 3 affected pigs and were submitted to the virology laboratory to be tested for the presence of swine influenza antigen using an antigen detection ELISA. **An influenza A virus was identified and, following virus isolation in eggs, was typed as an H1N1 virus using multiplex PCR**. Microscopic examination of lung tissue revealed attenuation, erosion and necrosis of epithelium in some bronchioles. This was accompanied by a marked neutrophilic reaction in the lumens of bronchi and bronchioles.

A piglet vaccination program for PRRSV was instituted immediately after the virus was identified in piglets from barn A, with piglets entering barn C being the first group to be vaccinated. Piglets in this barn broke with respiratory disease 3 weeks post-entry, with clinical signs similar, but slightly milder than those seen in barns A and B. The PRRSV vaccination program appeared to be successful since PRRS virus was not identified. **Serology confirmed H1N1 SIV as the key pathogen.** Aspirin was administered via drinking water, and clinical signs rapidly subsided. Even though the sow herds were not vaccinated against H1N1 SIV, respiratory problems have not been a concern in subsequent groups of nursery pigs, and production parameters have returned to previous levels.

Serum samples collected from sows of various parities from 2 of the sow herds revealed both to be unstable for PRRS virus, with the average mean and standard deviation being 0.907 ± 0.735 for Unit 1 and 1.626 ± 1.323 for Unit 2, using the PRRSV IDEXX antibody ELISA. Ten of 29 sows in Unit 1 and 12 of 28 sows in Unit 2, were identified as serologically positive for H1N1 SIV, using the SIV H1N1 IDEXX antibody ELISA. **Thus, both the PRRS virus and the influenza virus appeared to be derived from the sow herds. An SIV outbreak had not been identified in either of the sow herds**.

(continued on next page)

PRRS and swine influenza in a nursery

Continued from page 8

The severity of the respiratory outbreak in these nurseries was related to the combination of agents identified, and only by dealing with both of them was a successful outcome achieved.

The majority of swine influenza isolates recovered from Ontario swine by the AHL are classical swine H1N1 isolates. One H3N2 wholly human virus was recovered from a neonatal pig in 1997. Two H3N2 viruses, similar to the Colorado strain originally isolated from Colorado swine in 1977 and similar to the Quebec strain isolated in 1988, were identified in 1999. A totally avian H4N6 virus was isolated from one herd in 1999. The AHL has not yet isolated an example of the H3N2 triple reassortment (human/swine/avian) virus now prevalent in swine in the USA. Nor have we isolated an H1N2 recombinant virus, derived from the triple reassortment H3N2 virus and the classical H1N1 virus, that is now also circulating in swine in the USA.

From January 1998 to December 2002, we identified a total of 443 PRRS virus strains, using PCR for ORF7; 34 different RFLP cut patterns were identified within this total. Twelve percent (54) were untypable using standard PCR ORF5 RFLP typing. Sequence analysis of ORF5 gene products is available at the AHL, with 129 previously sequenced Ontario strains available for degree of homology comparisons, in addition to the virus sequences deposited in GenBank. *AHL*

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IDEXX HerdChek PRRSV 2XR antibody ELISA

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The AHL is now using the new PRRSV 2XR antibody ELISA, with which IDEXX has replaced their original PRRSV antibody ELISA. The new PRRSV 2XR antibody ELISA continues to detect antibody to both North American (NA) and European (E) strains of PRRSV. The new kit uses the same protocol, 0.400 cut-off sample-to-positive (S/P) ratio, and software for reporting. It is expected to have better consistency in performance. A comparison between the two kits, as provided by IDEXX, is included in the table below:

For more information on the new IDEXX PRRSV 2XR antibody ELISA, please contact Dr. Susy Carman at 519-824-4120 ext 54551, or *scarman@lsd.uoguelph.ca*

AHL

Table 1. Comparison of properties of IDEXX PRRSV ELISA kits

Kit type:	**New PRRSV 2XR antibody ELISA**	**Original PRRSV antibody ELISA**
Manufacturing method	Uses recombinant protein preparations for both NA and E strains of PRRSV	Used cell culture derived antigen preparation for NA strain; recombinant protein for E strain
Seroconversion (day post-infection)	Detected as early as day 8	Detected as early as day 9
Sensitivity using known positive sample sets	97.4%	100%
Specificity using known negative sample sets	99.5%	99.7%
Comparison of mean S/P ratios (slightly lower for same sample sets for PRRSV 2XR)	1.530 1.370 0.410 0.810 0.020 0.038	1.750 1.430 0.530 1.026 0.070 0.053
Single nonspecific reactors	Fewer expected	

HORSES

Equine proliferative enteropathy in 4 foals: An emerging cause of diarrhea and hypoproteinemia

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Sameh Youssef, Mihai Gagea, Beverly McEwen, Murray Hazlett, Tony van Dreumel

Between December 1, 2002 and January 24, 2003, 3 euthanized foals and 1 that had died (ages ranged from 7 to 11 months) were submitted for post-mortem examination with clinical histories of severe diarrhea, rapid weight loss, and hypoproteinemia. Proliferative enteropathy (PE) due to *Lawsonia intracellularis* infection was diagnosed based on pathology and polymerase chain reaction (PCR) assay. **The characteristic lesions were proliferative ileal (occasionally duodenal) adenomatosis** (Fig. 1), **sometimes ulcerated, containing intracellular argyrophilic bacteria typical of *L. intracellularis***. These are pathognomonic lesions of proliferative enteropathy, in horses and other species. However, the extension of the mucosal proliferation to the duodenum is unusual, and to the best of our knowledge has not been reported.

Lawsonia intracellularis is a recently identified bacterial pathogen that causes disease in many species. Very few equine cases have been reported, and all of these cases were in foals. From 1999 to January 2003, 11 cases of PE in horses have been diagnosed in our laboratory. Only 2 of these cases were from 1 farm.

Although pigs have been considered the major susceptible animal, it is becoming increasingly apparent that ***L. intracellularis* has a broader host range than previously thought**. Based upon 16S rRNA sequencing of amplicons from lesions from various species, there appears to be little genetic variation among organisms from one host species to another. Cross-infection from swine to equine or vice versa has not been reported.

Proliferative enteropathy should be included in the differential diagnosis of disease causing diarrhea in foals, especially when it is associated with severe hypoproteinemia. **The ante-mortem diagnosis of PE in foals is challenging**. Association of hypoproteinemia with diarrhea, combined with negative bacterial culture of other common bacterial enteropathogens in foals, increases the probability of PE. **Demonstration of acid-fast bacteria in the feces of infected foals is not accurate because a high percentage of false-negative results occur**. The sensitivity of a PCR test on feces is much lower than when done on ileal mucosal scrapings. Post-mortem diagnosis depends on the presence of the characteristic gross lesions and the pathognomonic histologic lesions; namely, intracellular colonization of epithelial cells by argyrophilic *L. intracellularis* with resultant epithelial hyperplasia. Confirmation of the diagnosis can be achieved by demonstration of bacteria using electron microscopy, immunohistochemistry, and a PCR test of ileal mucosal scrapings. *AHL*

Proliferative enteropathy should be included in the differential diagnosis of disease causing diarrhea in foals, especially when it is associated with severe hypoproteinemia

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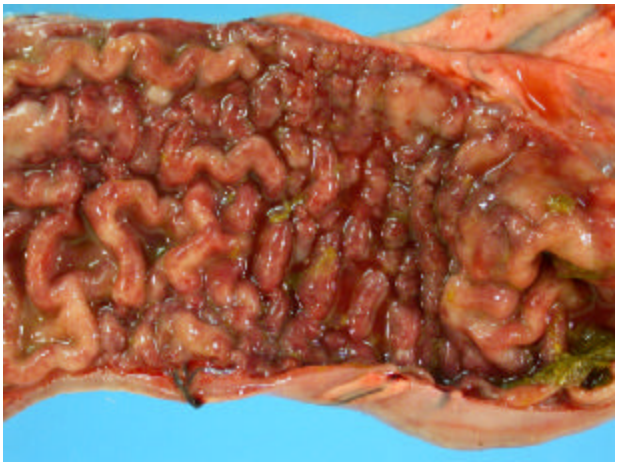

Figure 1. Ileum. Severely thickened distal ileal mucosa with nodular or ridge-like appearance.

Fatal equine herpesvirus infection in young horses

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We described in a preliminary report in the December 2002 AHL Newsletter a cluster of 3 young adult horses with suspected equine herpesvirus-1 (EHV-1) infection. They had been purchased from a common source or had contact with horses from this same source, and had a history of **acute onset of dyspnea and ataxia, progressing to death in less than 12 hours**. **Widespread lymphoid necrosis and pulmonary edema were the common necropsy findings**. EHV infection was confirmed by positive immunofluorescence for EHV-1/4 in the lung from 1 horse, and by immunohistochemistry with positive staining for EHV-1 in parenchymal cells and vascular endothelium of lymph node and lung in the other 2 horses. Such endotheliotropism is unusual in typical pulmonary EHV-1 infection in adult horses, although vascular targeting is important in the pathogenesis of the neurotropic form of the disease. Microscopically, viral inclusions consistent with herpesvirus were present in low numbers in lymph node, spleen, thymus, and in colonic crypt epithelium in 1 horse.

Two additional horses from this same common source were submitted for necropsy after being euthanized because of progressive ataxia that followed a mild upper respiratory tract infection. Histologically, these two horses had nonsuppurative myeloencephalitis consistent with a viral etiology, with the most prominent lesions present in white matter tracts of the spinal cord. Rabies and West Nile virus (WNV) infection were excluded by negative immunofluorescence and virus isolation (for rabies), and negative serology and immunohistochemistry results (for WNV). No virus was isolated from the CNS, lung, or lymphoid tissue of these horses, and immunohistochemistry for EHV-1 antigen was negative for spinal cord lesions. However, the character and distribution of histologic lesions, in combination with the clinical histories, were consistent with a diagnosis of neurotropic EHV-1 infection.

For cases of suspected EHV infection, it would be beneficial to attempt virus isolation from nasopharyngeal swabs early in the course of infection

The inability to isolate virus from appropriate tissues at the time of death in all 5 of these confirmed or suspected EHV-1 cases likely reflects the presence of virus-neutralizing antibody in blood, which normally develops during the course of infection. As occurred in the initial 3 horses, residual intracellular virus can sometimes be demonstrated using immunofluorescence or immunohistochemistry for a short time subsequent to the elimination of viremia by virus-neutralizing antibody. For cases of suspected EHV infection, it would be beneficial to attempt virus isolation from nasopharyngeal swabs early in the course of infection. *AHL*

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COMPANION ANIMALS

An overview of canine 'ehrlichiosis'

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Ehrlichiae are small, intracellular bacteria that infect blood cells in various animal species and humans. They replicate in membrane-bound vacuoles of specific types of host cells, chiefly granulocytes or monocytes. Ehrlichial organisms are vector-borne; they replicate in ticks and are transmitted from infected cells in vectors to the blood cells of animals or humans.

Classically, canine ehrlichiosis (Table 1) is seen as a non-specific multisystemic disorder characterized by depression, lethargy, mild weight loss, vomiting, diarrhea, and anorexia, with or without hemorrhage. Patients may also be presented with uveitis, retinal petechiae, polymyositis, polyarthritis, or central nervous system signs. Laboratory findings with canine ehrlichiosis include nonregenerative anemia, leukopenia, thrombocytopenia, hyperglobulinemia (monoclonal or polyclonal) and hypoalbuminemia.

Canine monocytic ehrlichiosis (*Ehrlichia canis)*

Canine monocytic ehrlichiosis (CME), caused by *E. canis*, is an acute to chronic disease of monocytes. This organism is primarily transmitted by *Rhipicephalus sanguineus*, the brown dog tick. Dogs may be presented with variable clinical signs including lethargy, anorexia and depression. **Thrombocytopenia** is the most consistent presenting abnormality and may be associated with hemorrhage, particularly epistaxis. Fever, uveitis, CNS signs or lymphadenopathy may also occur.

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An overview of canine ehrlichiosis - *cont'd from p 11*

Canine granulocytic ehrlichiosis

a) Canine granulocytic ehrlichiosis caused by ***Ehrlichia ewingii***, is a disease of **neutrophils** and, rarely, eosinophils. Infections usually cause mild signs, including fever, lethargy, anorexia, weight loss, vomiting, diarrhea, severe but transient thrombocytopenia, and transient mild nonregenerative anemia. The major presenting clinical signs may be lameness and swollen joints due to **polyarthritis**. Ticks including *Ixodes pacificus, Dermacentor variabilis, Rhipicephalus sanguineus, Amblyomma americanum* and *Ixodes scapularis* have been implicated as vectors.

b) Canine granulocytic ehrlichiosis caused by ***Anaplasma phagocytophilum*** is also associated with infection of **neutrophils and eosinophils**. Symptomatic infections are associated with pyrexia, anorexia, edema of limbs and CNS signs. Clinical pathology changes usually include leukopenia and thrombocytopenia. *Ixodes pacificus* and *Ixodes scapularis* appear to be the ticks that transmit this pathogen.

Canine infectious cyclic thrombocytopenia

Canine infectious cyclic thrombocytopenia (ICT), caused by ***Anaplasma (Ehrlichia) platys***, is characterized by **severe thrombocytopenia** (<15 X 10^9/L), occurring at regular intervals. The disease is often asymptomatic, but thrombocytopenic animals may hemorrhage after accidents or during surgery. Mild pyrexia, uveitis, petechiae and ecchymoses may be observed. The vector for this agent is not known, though *R. sanguineus* is suspected.

Since 1998, 16 out of 206 sera from dogs in Ontario were seropositive for *E. canis*. At least 12 of these animals had clinical signs compatible with ehrlichiosis. Furthermore, of the symptomatic animals, 2 had traveled to Africa, 1 to Costa Rica and 1 to Venezuela. The travel history for the other animals was unknown. Serology for other *Ehrlichia* species was rarely requested. Unfortunately, the prevalence of the different *Ehrlichia* species in Ontario dogs is not known. However, on the basis of the relative abundance of the various tick vectors, it would be prudent to request serology for both *A. phagocytophilum* (*E. equi)* and *E. canis*. Some diagnostic laboratories also offer PCR for *Ehrlichia*. For all of these tests, practitioners should be fully informed of their specificity. Furthermore, practitioners should appreciate that many *Ehrlichia* infections in dogs are asymptomatic. **A diagnosis of ehrlichiosis depends on both a positive *Ehrlichia* test and clinical signs consistent with infection.** *AHL*

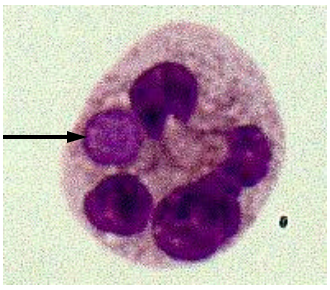

Figure 1. Ehrlichia inclusion (arrow) in a segmented neutrophil from the cerebrospinal fluid of a dog infected with an *Ehrlichia spp.*

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Table 1. *Ehrlichia/Anaplasma* species that most commonly infect dogs in North America

Old name	Current name	Distribution
Ehrlichia canis	***Ehrlichia canis***	Uncommon in Ontario, more common in southern USA
Ehrlichia ewingii	***Ehrlichia ewingii***	Rare in Ontario, more common in southern USA
Ehrlichia equi	***Anaplasma phagocytophilum***	More common in western USA, Canada unknown
Ehrlichia platys	***Anaplasma platys***	Southern USA only

Questions, comments, suggestions for the editor??

- Questions about services provided by the AHL?
- Suggestions for topics to be covered in future editions of the Newsletter?

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