In this issue:

AHL Christmas hours, 2001

The AHL will be **open with limited staffing 9 AM - 5 PM, December 22, 24, 27, 28, 29, 31.** Emergency necropsy service is available in Guelph on all holidays except Christmas Day.

Check out our website! **http://ahl.uoguelph.ca**

- ✓ How to pack and ship submissions
- ✓ All issues of the AHL Newsletter
- ✓ Fee Schedule, test index
- ✓ User's Guide
- ✓ Maps/directions to our labs
- ✓ LabNotes
- ✓ Staff directory
- ✓ Quality program, test validation data
- ✓ Mastitis forms
- ✓ Links to West Nile virus information
- ✓ and more!

And it's searchable too!

The AHL has again successfully passed the **Johne's proficiency check test** administered by the National Veterinary Services Laboratory of the USDA.

Season's Greetings from the staff of the Animal Health Laboratory

AHL Newsletter
December 2001 - Volume 5, Number 4
Editor: **Dr. Grant Maxie**
Editorial Assistant: **Ms. Helen Oliver**

The *AHL Newsletter* is published quarterly (March, June, September, and December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph. Its mission is to inform clients and partners of the AHL of current AHL activities, animal disease events, and disease trends.

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Comments? Suggestions?
If you as a client or partner of the AHL have comments or suggestions, or would like to be added to, or removed from, the AHL Newsletter **mailing list**, please contact **Ms. Helen Oliver** at:

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GENERAL

Escherichia coli genotyping is now available at the AHL

Hugh Cai, Marie Archambault, Leah Read

We offer a genotyping assay based on detection by PCR of the genes of K88, K99, 987P, F18, F41, STa, STb, LT, and STX2e. Nine PCR tests are combined into two multiplex PCR's to reduce the cost to the client and to decrease turnaround time - we are very pleased to have had the assistance of Dr. Carlton Gyles in transferring this method to our lab.

The fee is $25/sample for food animals, and $50/sample for non-food animals. Please forward both your request and your sample to the AHL bacteriology laboratory, as genotyping is performed on *E. coli* cultures.

For enquiries about the test, please contact the AHL at 519-824-4120 for Dr. Marie Archambault (ext 4536), Dr. Hugh Cai (ext 4316) or Leah Read (technologist, ext 4086).

Enterotoxigenic *E. coli* (ETEC) diarrhea occurs in neonatal and weaning pigs, calves, and lambs; infections have also been reported in dogs and horses. ETEC may contain one or more adhesins capable of attaching to epithelial cells, termed K88, K99, 987P (also named F4, F5 and F6, respectively), and F41, which possess some host species specificity - K88 and K987P are almost always associated with isolates from swine; K99, from cattle, sheep and swine; and F41, from cattle. Besides adhesins, ETEC strains also produce one or more enterotoxins, including heat-labile toxin (LT) and heat-stable toxins (STa, STb).

Edema disease, an acute, often fatal, enterotoxemia of weaned pigs, is characterized by subcutaneous and subserosal edema, caused by absorption of a shiga-like toxin STX2e produced by certain types of *E. coli.*

For information on **verotoxigenic *E. coli*** (VTEC) infections, please refer to the AHL Newsletter, June 2001, pp. 25-26.

Reference

Bosworth BT, Casey TA. Identification of toxin and pilus genes in porcine *E. coli* using multiple primer pairs via PCR. 97th Gen Mtg Am Soc Microbiol 1997.

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Zoonotic pathogens and diseases identified at the AHL, 1998 - 2001

Beverly McEwen, Marie Archambault, Davor Ojkic, Tony van Dreumel, Gary Thomson, Peter Lusis

Recent epidemics of avian influenza in Hong Kong, new-variant Creutzfeldt-Jacob disease in the UK, and fatal Hendravirus infection in Australia are reminders that **many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals**. These diseases are added to the already established list of rabies, leptospirosis, brucellosis, salmonellosis, toxoplasmosis, etc. that continue to affect human and animal populations globally. Domestic and wild animals act as reservoirs for these pathogens, which may be viruses, bacteria, parasites, or prions. The importance of the relationship among livestock diseases, human health, and society in general is well stated in a recent article in *Science* (1).

Zoonotic agents are acquired through foodborne/ waterborne routes, contact of individuals with infected animals, by arthropod vectors, or via the environment. Veterinarians, farmers and abattoir workers may be exposed at work. Immunosuppressed people and young children are at higher risk of acquiring zoonotic infections, and pregnant women are susceptible to certain pathogens.

The AHL plays an important role in public health by identifying pathogens common to animals and people. 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.** 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.

Pregnant women should be particularly cautious about exposure to potential sources of *Listeria monocytogenes*, *Toxoplasma gondii, Chlamydia psittaci,* and *Coxiella burnetii.* Chinchillas represented a large proportion of species listed as 'other' with *L. monocytogenes* in Table 1.

Although not evident from Table 1, veterinarians should be aware that **companion animals can be important sources of zoonotic infections**. Examples include exposure to cats infected with *Chlamydia psittaci*, or parturient cats infected with *Coxiella burnetii*.

Serological identification of ***Leptospira sp.* infection** is necessary as leptospires are difficult to isolate. The AHL is currently developing PCR tests to

Table 1. Zoonotic pathogens isolated and/or identified at the AHL, 1998 – September 2001.

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	Jan-Sept 2001	2000	1999	1998
Bacillus anthracis[1]											0	0	0	0
Campylobacter coli/ jejuni/fetus subsp. fetus	1		1	2				5	3	6	18	14	6	11
Chlamydia psittaci				6	5					1	12	21	14	13
Coxiella burnetii (Q fever)				1	3						4	4	4	2
Cryptosporidium sp.	148	2	1	1	5			1		2	160	120	93	127
Francisella tularensis										3	3	6	0	0
Giardia sp.	5	1			3			7	3		19	26	4	14
Listeria monocytogenes	15				7					12	34	34	16	22
Mycobacterium bovis[1]											0	0	0	1
Rabies[1]	2		1	1				1			5	1	1	0
Salmonella sp [2]	79	151	49	3		10	31		5	32	360	754	711	671
Streptococcus suis	43	336		1							380	560	489	664
Streptococcus equisimilis		66	14	1				6		1	88	154	93	110
Streptococcus zooepidemicus	11	1	125	1							138	162	220	134
Toxoplasma sp				12							12	8	13	15
Verotoxigenic *E. coli*[3]											0	3	1	1
Yersinia enterocolitica	1	2								1	4	8	2	9

1 Federally reportable diseases confirmed by the Canadian Food Inspection Agency

2 *Salmonella sp* isolated Jan-June 2001; 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)

improve diagnosis of this important zoonotic pathogen in domestic animals.

Infrequently identified pathogens can have profound public health impact. In 2001 alone, one owner was killed by his **rabid horse** (see AHL Newsletter, March 2001, p.14) and several children had to receive prophylactic treatment after their unvaccinated dog was rabies positive. The last case of **anthrax** identified at the AHL was in the Kemptville laboratory in 1996 in two dairy cows found dead on a farm near a recently excavated site. About 65 years previously, cattle had died of anthrax on the same farm.

Reference

1. Zinsstag J, Weiss MG. Livestock diseases and human health. Science 2001; 294: 477.

Table 2. Number of *Leptospira sp*. seropositive samples (>1:160) identified at AHL, 1998 – September 2001, by the microscopic agglutination test.

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Feline	Jan-Sept 2001	2000	1999	1998
L. autumnalis				15	1	16	50	2	2
L. bratislava		51	29	14		94	88	116	22
L. grippo-typhosa		12	5	14		31	32	18	61
L. hardjo	20					20	7	16	1
L. icterohaem-orrhagiae	232		1	8	1	242	140	224	31
L. pomona	52	1	14	7		74	112	152	40

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Antimicrobial-resistant bacteria isolated by the AHL from small animals and horses, 1998-1999

Marie Archambault, Beverly McEwen, John Prescott

Knowledge of the percentage resistance of bacteria commonly isolated from pet dogs and cats, and from horses (Table 1), may be of use to practitioners in drug selection, as well as providing a benchmark against which to measure changes in resistance over time. We recognize that this represents a "worst case scenario" since bacteria isolated from antimicrobially treated animals are usually more resistant than bacteria isolated from untreated animals. The comparison of antimicrobial resistance in common bacteria isolated from horses to those isolated from dogs and cats is instructive because of some differences in antimicrobial resistance. These correlate to differences in drug use between species.

Among points to note are:

- The high prevalence of susceptible *Streptococcus* species in pets (except to tetracycline), and in horse *S. zooepidemicus* (except to amikacin). **Streptococci have, in comparison to many other pathogens, remained highly susceptible to many antibiotics.**
- The emergence of **enrofloxacin resistance (20%) in pet *E. coli*** compared to 2% of *E. coli* from horses, in which the drug is not used.
- The 16-18% resistance to clindamycin and erythromycin in **pet *S. intermedius*** but virtually no resistance to cephalothin, clavamox, and oxacillin.
- The high level of **equine *E. coli* resistance to trimethoprim-sulfamethoxazole** (TMS), which may be used more in horses than in pets.
- The large number of ***Enterococcus* species** isolated from dogs; some species are naturally resistant to many common antimicrobials. This genus may also be increasing in frequency of isolation.

Table 1. Percent resistant bacteria isolated from small animals and horses by the AHL, 1998-1999.

Organism [number of isolates]	**AK**	**CLI**	**ERY**	**EN**	**AMP**	**CLA**	**CEP**	**PEN**	**OXA**	**GEN**	**TET**	**TMS**	**CH**
Staphylococcus intermedius (pet) [144]	1	16	18	6	24	0	3	30	4	1	29	7	1
Proteus spp. (pet) [63]	0	100	96	11	27	9	11	64	98	11	92	14	16
Streptococcus spp. (pet) [74]	2	7	5	1	1	0	3	3	3	0	44	5	0
Enterococcus spp (pet) [106]	77	96	31	22	24	22	85	56	97	39	48	19	13
Pseudomonas aeruginosa (pet) [115]	4	99	99	30	93	97	98	99	99	8	90	61	90
Escherichia coli (pet) [270]	0	100	100	20	38	22	22	100	100	6	21	10	17
Escherichia coli (horse) [99]	1	97	98	2	49	14	26	98	95	8	40	46	21
Actinobacillus equuli (horse) [56]	16	26	9	3	16	0	2	32	16	0	5	17	0
Streptococcus zooepidemicus(horse)[148]	51	-	1	6	4	0	1	4	2	8	42	38	0

AK, amikacin; CLI, clindamycin; ERY, erythromycin; EN, enrofloxacin; AMP, ampicillin; CLA, amoxicillin-clavulanate; CEP, cephalothin; PEN, penicillin; OXA, oxacillin; GEN, gentamicin; TET, tetracycline; TMS, trimethoprim-sulfamethoxazole; CH, chloramphenicol.

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CATTLE

Outbreak of clinical leptospirosis in a dairy herd

Margaret Stalker, Brent Hoff, Frits Verzijlenberg

Six animals from a closed, unvaccinated, 68-head dairy herd developed clinical signs of fever and hematuria three weeks after being turned out onto pasture. Four of the affected animals died. Three cows subsequently aborted.

Samples submitted to the AHL included an **EDTA blood sample with grossly visible hemolysis.** A CBC revealed **marked anemia**, with a red cell count of 1.2 x 10^{12}/L (reference range 4.9-7.5 x 10^{12}/L), hemoglobin of 25 g/L (reference range 84-120 g/l) and hematocrit of 0.06 L/L (reference range 0.21-0.30 L/L). There was also leukocytosis with a left shift. Abnormalities in a bovine serum chemistry profile included marked elevation of total and free bilirubin levels, and mildly elevated urea.

A leptospira serology screen of a single acute serum sample revealed a titer of 1:1280 for *Leptospira pomona*, 1:320 for *L. icterohaemorrhagiae*, and <1:40 for *L. hardjo*. Subsequent acute and convalescent serum samples from seven cows showed seroconversion to both *L. pomona* and *icterohaemorrhagiae* in three, and to *L. icterohaemorrhagiae* in a fourth animal.

Histologic changes included peracute periacinar hepatic degeneration, likely secondary to hypoxia associated with the marked anemia/hemolysis, and mild acute nephrosis with low numbers of mononuclear cells infiltrating the renal interstitium. **Special stains** of the kidney and liver (Warthin-Starry silver stains) revealed numerous spirochetes, often with a hook at one end, morphologically consistent with leptospires. The identity of these organisms was confirmed by **immunoperoxidase staining** with antibodies specific to the leptospira group. The AHL **PCR test** for leptospires (currently validated for urine samples only) was strongly positive for both liver and kidney.

Clinical signs of acute leptospirosis include septicemia with a high fever, depression, mucosal petechiae, and acute hemolytic anemia with hemoglobinuria, jaundice and pallor. Case fatality can be high. Calves under a month of age are most susceptible. **This case is unusual as adult cattle are more typically affected with a subacute or chronic form of the disease.** Cows with **subacute disease** may have a mild fever, depression, anorexia, hemoglobinuria and often abort or exhibit a marked decrease in milk production associated with a soft udder and yellow-orange or blood-stained secretion. A **chronic form of the infection** also occurs, associated with late-term abortion and seroconversion, often without clinical illness. These infected animals continue to shed leptospires in their urine, and are an important undetected source of infection for the rest of the herd. In this case, a herd vaccination program was implemented to control spread of the disease.

Leptospirosis is also an occupational hazard for veterinarians and livestock producers. The human disease resembles influenza, with fever and severe headaches; serious complications may develop in some cases. Leptospirosis is difficult to distinguish from many other diseases in humans and might not be suspected unless the medical practitioner is advised of the person's occupational risk.

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POULTRY

Ontario broiler, broiler breeder, layer, and turkey disease summaries for 2000-2001

Brian Binnington, Emily Martin, Beverly McEwen

Yearly summaries of specific disease entities are produced from the AHL database of pathology diagnoses made at the Guelph and Kemptville AHL labs. The following are some highlights of the more frequently diagnosed conditions or diseases of broilers, broiler breeders, layers, and turkeys.

Note: The submissions to the Animal Health Laboratory are a subset of Ontario cases. These numbers are based on pathology diagnoses and should be used for general trends only.

Broiler chickens (Table 1)

Bursal lymphoid depletion was a common finding in broilers, from 3 weeks to market age, submitted to the AHL. Although a number of factors can cause bursal lymphoid depletion, it is likely that IBD viral challenge is frequently involved. The AHL is currently implementing molecular techniques that should provide information on the presence of IBDV and strains of the IBDV that are associated with bursal lymphoid depletion. Also, **inclusion body hepatitis** continues to be prevalent. We observed decreased numbers of **infectious bronchitis** cases in 2000-2001.

Table 1. Broiler chicken pathology diagnoses, AHL labs, fiscal years (May 1 - April 30).

Pathology diagnosis	98-99	99-00	00-01	May - Sept 2001
Enteritis, coccidia	43	29	35	4
Enteritis, necrotic	22	13	6	2
Enteritis, other	0	2	2	0
Hepatitis, inclusion body	19	23	21	7
Hepatitis, other	17	14	14	1
Arthritis, bacterial	41	24	17	2
Arthritis, viral	1	1	1	0
Rickets	17	13	6	1
Long bone deformities	20	15	15	0
Tibial dyschondroplasia	27	36	19	1
Spondylolisthesis (kinky back)	2	4	3	0
Ascites	52	30	28	3
Acute death syndrome	36	37	26	2
Spiking mortality	2	12	15	2
Yolk sacculitis	37	35	52	5
Omphalitis	19	30	17	0
Starveout/dehydration	16	8	12	1
Septicemia, *E. coli*	61	62	61	4
Septicemia, salmonellosis	3	9	7	1
Septicemia, unidentified	34	22	12	3
Cellulitis	9	8	5	0
Botulism	4	6	4	2

Broiler breeders (Table 2)

Bacterial infections were the most frequent diagnoses in 2000-2001. In order of frequency, ***E. coli,*** ***Staphylococcus*** and ***Pseudomonas*** were the most common organisms isolated from **bacterial septicemias** in young birds. In older birds, ***E. coli*** and ***Staphylococcus*** were the two most commonly isolated organisms from bacterial septicemias. ***Staphylococcus*** and ***E. coli*** were also the two main organisms isolated from **arthritis/tenosynovitis** cases. There has been an apparent decrease in **fatty liver/hemorrhage syndrome** cases in 2000-2001.

Table 2. Broiler breeder chickens, pathology diagnoses, AHL labs, fiscal years (May 1 - April 30).

Pathology diagnosis	98-99	99-00	00-01	May - Sept 2001
Septicemia	13	15	10	4
Septicemia, salmonellosis	0	0	0	0
Arthritis/tenosynovitis	12	11	10	6
Osteomyelitis	2	3	4	3
Tendon rupture	1	1	1	1
Cellulitis	3	3	2	0
Pododermatitis	3	0	2	2
Pneumonia, bacterial	4	6	0	0
Peritonitis (yolk, bacteria)	2	14	2	1
Enteritis, coccidia	4	2	1	0
Enteritis, necrotic	3	5	2	0
Osteomalacia/hypocalcemia	2	1	1	1
Hepatitis	6	1	0	3
Fatty liver/hemorrhage syndrome	5	4	1	0
Neoplasia, leukosis complex	1	1	2	0
Neoplasia, lymphoid leukosis	1	4	1	0
Neoplasia, myelocytomatosis	5	5	6	1
Neoplasia, Marek's	0	0	0	0

Layers (Table 3)

As in previous years, **nutritional and metabolic diseases** and **bacterial septicemia** were the most frequent diagnoses. **Fatty liver/hemorrhage syndrome** declined, as for broiler breeders, but calcium insufficiency continues to cause losses through acute death due to **hypocalcemia** or inadequate bone strength (**osteomalacia - cage layer fatigue**). Production drops associated with **infectious bronchitis virus** were less frequently identified in 2000-2001.

Table 3. Layer chickens, pathology diagnoses, AHL labs, fiscal years (May 1 - April 30).

Pathology diagnosis	98-99	99-00	00-01	May - Sept 2001
Neoplasia, Marek's	4	8	4	1
Neoplasia, leukosis complex	1	0	1	0
Neoplasia, lymphoid leukosis	1	2	2	0
Neoplasia, other	0	1	0	0
Osteomalacia (cage layer fatigue/hypocalcemia)	15	20	15	2
Nephrosis/visceral urate deposits	5	6	7	2
Hepatitis/splenomegaly (hemorrhagic necrotizing hepatitis)	1	6	6	1
Fatty liver/hemorrhage syndrome	15	14	4	0
Peritonitis (yolk, bacteria, etc.)	7	9	3	2
Enteritis, coccidia	6	3	7	2
Enteritis, necrotic	6	2	7	2
Fowl pox	0	0	0	0
Northern fowl mite	0	1	0	0
Septicemia, *Salmonella*	0	0	0	0
Septicemia, other	5	16	14	0
Infectious bronchitis virus	8	7	3	0

Turkeys (Table 4)

Bacterial infections continued to be the most common cause of mortalities in post-mortem submissions this year. There has been a continued

decline in the number of cases of ***E. coli* septicemias** since 1996-97.

Table 4. Turkeys, pathology diagnoses, AHL labs, fiscal years (May 1 - April 30).

Pathology diagnosis	98-99	99-00	00-01	May - Sept 2001
Enteritis, unspecified	4	2	7	0
Enteritis, coccidia	1	2	7	3
Enteritis, hemorrhagic	0	0	0	0
Enteritis, parasitic	1	2	0	1
Enteritis, necrotic	4	3	8	2
Enteritis, *Salmonella*[a]	-	-	5	1
Hepatitis, other	4	1	2	0
Histomoniasis	2	2	2	0
Mycotic/aspergillosis	4	9	7	0
Candidiasis	7	3	11	0
Septicemia, *E. coli*	30	27	19	1
Septicemia, salmonellosis	5	24	13	0
Yolk sacculitis/omphalitis	8	12	13	2
Starveouts	9	15	5	1
Arthritis	4	3	11	2
Rickets	3	1	1	0
Long bone deformities	2	5	4	0
Pododermatitis	2	1	1	1
Round heart (STC)	8	10	6	1
Myopathy	5	4	2	4
Botulism	0	0	0	0
Erysipelas	4	2	2	0
Fowl cholera	1	0	0	0
Ornithobacterium rhinotracheale	2	4	0	0
Bordetella avium	3	1	0	0
Coronavirus, enteric	2	0	0	0

[a] = disease not previously enumerated

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New *Salmonella enterica, Salmonella* Enteritidis, *Salmonella* Typhimurium PCR's

We have developed specific and sensitive PCR assays for screening pure cultures and poultry house samples:

- *Salmonella/Pseudomonas* multiplex PCR
- *S. enterica* serotype Enteritidis PCR
- *S. enterica* serotype Typhimurium *mdh* PCR

Complete validation data are posted on our website: http://ahl.uoguelph.ca/quality/

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SWINE

Erysipelothrix isolations increased at the AHL in 2001

Gaylan Josephson, Marie Archambault, Beverly McEwen

Erysipelas outbreaks are associated with, among other events, environmental stresses, particularly sudden changes in temperatures as occurred during extended hot periods this past summer. A review of *Erysipelothrix rhusiopathiae* isolations made at the AHL indicates that this organism is isolated from clinical cases throughout the year. **We have noted an increase of isolates in 2001 over the previous several years** (Table 1).

Because this syndrome is easily diagnosed in the field, laboratory submissions related to the skin form of erysipelas are virtually non-existent - samples are submitted only when the practitioner needs microbiological confirmation that *Erysipelothrix rhusiopathiae* is present. Thus, the number of diagnoses made at a diagnostic laboratory are not representative of the actual incidence of clinical erysipelas in the industry. Given the submission bias to the AHL and the very low numbers, this trend cannot be directly extrapolated to the industry, although swine practitioners have suggested that they saw an increase in cases of clinical erysipelas during the past year.

Of interest is the number of cases in which the organism was isolated from suckling piglets. Infection in this age group is a direct reflection of the immune status of the dam, since colostrally derived maternal antibodies are protective during this period. A review of these cases identified that multiple losses often occurred within individual litters. In many cases, producers reported a coinciding outbreak of the diamond skin form of the disease in sows. In six cases, gilt litters only were involved, suggesting that a review of the gilt vaccination program was in order.

Questions frequently asked by practitioners and producers alike concern possible genetic change or serotype variation of the bacterium, and to a possible interaction with PRRS virus. There has been no evidence that either of these factors are involved. Rather, **the short duration of immunity following vaccination, and the production changes that have occurred in the swine industry, may have been the main contributing factors to this apparent increase in erysipelas**. In any case, following a case of clinical erysipelas in a swine herd, the vaccination program should be closely reviewed and possibly altered.

Table 1. Age of pigs from which *Erysipelothrix rhusiopathiae* was isolated at the AHL, 1991-2001.

Year	Suckling pigs	Nursery	Grow	Finish	Adult	Total no. of isolates
2001 (Jan-Nov)	6	1	1	1		9
2000	2					2
1999	1			1		2
1998						0
1997	2			1	1 - abortion	4
1996	5	1	1	2		9
1995	2			3	1	6
1994	1		2	2		5
1993	2	1	1	1		5
1992	5		1	1		7
1991	2	1		2		5
Total	**28**	**4**	**6**	**14**	**2**	**54**

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Monitoring boar semen for PRRSV using a nested PCR

Susy Carman, Hugh Cai, Gaylan Josephson

For boar monitoring programs, the AHL now offers a nested PCR test for detection of PRRS virus in semen. This nested PCR test can identify 0.005-0.05 $CCID_{50}$/mL of semen. This is a 100- to 1000-fold enhancement above our routine PCR offered for serum and tissues, which finds 5-10 $CCID_{50}$/mL of serum or gram of tissue.

The fee is $26 per test.

Please ask specifically for this "**nested PRRSV PCR**" for evaluation of your samples, otherwise we will use the routine, less expensive, PRRSV PCR.

Monitoring for PRRS virus in boar studs has become an important control measure to prevent the spread of PRRSV into non-infected swine herds. Programs to maintain PRRSV-negative boar studs should encompass excellent biosecurity, quarantine periods, and diagnostic testing. Diagnostic testing needs to include both a serological monitoring program using the PRRSV IDEXX HerdChek ELISA and planned evaluation of semen using PCR (1). *In comparison to tissues and serum, much smaller quantities of PRRSV are present in semen, making it better to use the most sensitive PCR test available.*

Nested PCR tests are more sensitive than routine PCR tests. However, there is a trade-off.....they are more expensive to run, take longer to complete, and have a higher potential for laboratory contamination. Despite these potential disadvantages, semen is better evaluated using the more sensitive nested PCR.

References

1. Christopher-Hennings J. Monitoring for porcine reproductive and respiratory syndrome virus (PRRSV) in the boar stud. J Swine Health Prod 2001; 9: 186-188.

Validation of IDEXX H1N1 swine influenza virus ELISA

Susy Carman, Beverly McEwen, Gaylan Josephson

The IDEXX H1N1 swine influenza virus (SIV) ELISA is now offered by the AHL as a valuable reproducible test for **monitoring swine herds for the presence or absence of SIV H1N1 antibody**. Please note that this test is *not* intended to evaluate sera for antibody to H3N2 SIV strains.

Test results for the IDEXX H1N1 ELISA will be reported in the same manner as for the PRRSV IDEXX ELISA, including both S/P ratios and titer groups for individual animals and geometric mean calculations for specified collections of sera.

The fee is $6 per test.

Details of validation:

We validated the IDEXX H1N1 SIV ELISA by:

- determining sensitivity and specificity using sera collected from 345 pigs of known SIV-negative antibody status (pre-vaccination) and sera collected from the same pigs 2 weeks following the second vaccination with a killed SIV vaccine, and
- comparing the H1N1 SIV ELISA with the standard H1N1 hemagglutination-inhibition (HI) test.

The IDEXX H1N1 SIV ELISA was sensitive (98.8%, 95% CI 96.9 - 99.6) and specific (91.6%, 95% CI 88.0 - 94.2) for the identification of antibody-negative sera from unvaccinated animals and antibody-positive sera from vaccinated animals. At a titer cut-off of 1:8, the HI test had similar sensitivity (97.4%, 95% CI 95.1 - 98.9) but significantly lower specificity (74.2%, 95% CI 69.2 - 78.7). Changing the HI cut-point did not result in an overall improvement in the accuracy of the HI test at low antibody level.

There was poor correlation of either the S/P ratio or the titer group of the SIV ELISA compared to the HI titer in pigs post-vaccination. No correlation could be found for an incremental increase in either the SIV ELISA titer or titer group and a 4-fold or more increase in HI titers. **Therefore, the SIV ELISA cannot be used to replace the HI test for the evaluation of "paired sera" for the serological diagnosis of SIV infection or disease.**

Complete validation data are posted on our website: http://ahl.uoguelph.ca/quality/

Contact: scarman@lsd.uoguelph.ca

HORSES

Fatal *Strongyloides westeri* infection in a diarrheic foal

Peter Lusis, Ron Ford

A 4-month-old Standarbred foal died after prolonged diarrhea ('since he was born' according to the owner) and stunted growth. His appetite was good, but weaning (at 3 months of age), diet and bedding changes, and various treatments (Strongid T, blood fortifiers, penicillin, cimetidine) did not improve his condition.

On gross postmortem examination, the foal was in thin body condition. The stomach was impacted with dry grain and roughage, and contained moderate numbers of bots. The small intestine contained a few ascarids and abundant clear mucus with small amounts of partly-digested roughage and grain. The cecum and colon contained semi-solid digesta with a small quantity of formed feces in the rectum. No other significant lesions were evident grossly.

Large numbers of *Strongyloides westeri* ova were present on fecal flotation, and numerous nematodes were present in sections of the upper to mid small intestine. No significant bacteria were isolated.

Although many animals with *S. westeri* infections are asymptomatic, the parasite can cause severe diarrhea and death in some foals. **Foals can be infected through mare's milk or colostrum (most common), ingestion of larvae on feed, or percutaneously**. Onset of diarrhea can occur as early as 10-14 days of age in some foals, and because of a short pre-patent period (5-7 days), infected foals can be passing many *S. westeri* ova at 2 weeks of age. Strong immunity develops in most foals by 4-6 months of age.

Ivermectin and benzimidazole anthelmintics are effective against *S. westeri*. Mares should be dewormed within 24 hours after parturition to minimize foal exposure. Treating foals at 2 and 4 weeks of age and every 4 weeks thereafter may limit infections, but may delay the onset of immunity and prolong susceptibility to reinfection. Except in clinical cases, treatments at 4 and 12 weeks of age may be preferable.

Reference

Merck Veterinary Manual, 8th edition. 1998: 205, 1480.

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Streptococcus equi PCR is now available at the AHL

Hugh Cai, Marie Archambault, Leah Read

Streptococcus equi is the causative agent of **equine strangles**, which continues to be one of the most important respiratory diseases of horses. The AHL has validated and implemented a PCR method that amplifies the SeM gene of *S. equi* and can be used to confirm the identification of *S. equi* isolates (1).

The fee is $32/sample.

Please forward both your request and your sample to the bacteriology laboratory, as the test is performed only on pure cultures at this time. Atypical isolates of *S. equi* occur and might be misidentified biochemically.

For enquiries about the test, please contact the AHL at 519-824-4120 for Dr. Marie Archambault (ext 4536) or Dr. Hugh Cai (ext 4316).

Reference

1. Timoney JF, Artiushin S. Detection of *Streptococcus equi* in equine nasal swabs and washes by DNA amplification. Vet Rec 1997; 141: 446-447.

Ehrlichia risticii PCR test validation - limited-time, free testing!

Hugh Cai, Marie Archambault, Leah Read

Ehrlichia risticii is the cause of **Potomac horse fever**, an acute equine diarrheal syndrome consisting of fever, listlessness, anorexia, leukopenia, diarrhea and laminitis. Early diagnosis was difficult, until a PCR test was developed. The AHL has analytically validated a published *E. risticii* PCR test (1). **We will accept samples from suspect Potomac horse fever cases for free testing until we finish field validation**.

Please submit **15 mL EDTA blood** from the suspect case with a request on the submission form for the "**no-charge *Ehrlichia risticii* PCR test**".

To enquire about the test, please contact the AHL at 519-824-4120 for Dr. Marie Archambault (ext 4536) or Dr. Hugh Cai (ext 4316).

Reference

1. Biswas B, Mukherjee D, Mattingly-Napier BL, Dutta SK. Diagnostic application of polymerase chain reaction for detection of *Ehrlichia risticii* in equine monocytic ehrlichiosis (Potomac horse fever). J Clin Microbiol 1991; 29: 2228-2233.

Contact: hcai@lsd.uoguelph.ca

COMPANION ANIMALS

Two outbreaks of feline panleukopenia in cats from animal shelters

Margaret Stalker, Murray Hazlett, Susy Carman

Two animal shelters submitted 5 young cats for post-mortem examination at AHL-Guelph. **The clinical history was of apparently healthy cats, or cats exhibiting mild upper respiratory signs, deteriorating and dying over the course of 24 to 48 hours, with minimal clinical signs.**

Post mortem findings were subtle, and restricted to the intestinal tract in 4 of the 5 cats, with either contraction or focal dilation of small intestine, mild subserosal edema, patchy mild petechiation of the serosal surface, and in one case fibrin in the intestinal lumen. One kitten also had bronchopneumonia, from which *Bordetella bronchiseptica* was cultured. Histologic changes in intestinal sections were much more dramatic, with severe diffuse crypt epithelial necrosis and villus collapse, typical of feline panleukopenia (Figure 1).

Fluorescent antibody testing and immuno-peroxidase staining of sections of gut from all cats were positive for feline parvovirus (FPV), while parvovirus antigen ELISA's were positive in 3 of the 5 cats. FPV was isolated from two of the three ELISA-positive cats. No herpesvirus or calicivirus was identified by virus isolation or immunohistochemistry from any of the cats.

FPV is a ubiquitous virus that is highly contagious and persistent in the environment. Unvaccinated kittens acquire maternal immunity through colostrum, and are usually protected for up to
3 months of age. Susceptibility increases as maternal immunity wanes, and severe clinical illness can occur in young unvaccinated kittens, with the highest morbidity and mortality between 3 and 5 months of age.

While the **acute disease**, characterized by fever, anorexia, vomiting, dehydration and variable diarrhea, is more common, **peracute disease**, characterized by rapid development of endotoxemia associated with the damaged intestinal mucosa, is not unusual. Cats may die from septic shock within 12 hours, with few or no premonitory signs. **Post mortem lesions in cats with peracute disease are often minimal**. Antigen ELISA's used to detect parvovirus in feces or intestinal contents may be negative in these cases, either due to the short period of actual shedding of virus in feces, or due to low levels of circulating antibodies, which leak from the vasculature of the

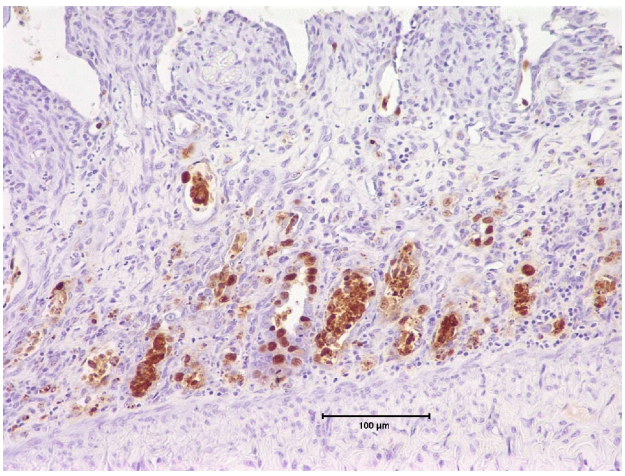

Figure 1. Small intestine with severe crypt necrosis and positive immunoperoxidase staining (brown dots and clumps) for feline parvovirus/panleukopenia.

damaged gut mucosa and bind to the virus, reducing the sensitivity of the ELISA. **A negative parvovirus antigen ELISA does not rule out parvovirus infection.**

Feline panleukopenia remains a primary differential diagnosis for sudden death in unvaccinated young cats with minimal gross post mortem changes.

The present cases had received considerable press coverage due to concern about a particularly virulent strain of feline calicivirus that was seen in several animal shelters in southern New York state, Massachusetts, and Pennsylvania. Facial edema as well as edema of the extremities and skin ulceration were clinical features, and the mortality rate was reported to be high (personal communication, Dr. James R Richards, Cornell Feline Health Center).

Veterinarians should also be aware that calicivirus is easily and commonly cultured from cats, with about 25% of cats being carriers. **Identification of calicivirus by isolation or other means does not necessarily mean that it is of clinical significance**.

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Outbreak of hepatotoxicosis in sled dogs

Brent Hoff, Peter Lusis

Twenty-six of 381 sled dogs became ill, and one was euthanized. **The problem was encountered in two different locations within a short time of consuming a ration of fortified mink feed.** Some dogs refused feed but resumed eating when the mixed grain cereal component was changed.

Clinical signs included inappetence, loss of condition, and severe jaundice. Significant clinical pathology findings were increased activity of serum hepatic enzymes (ALT, AST and SAP), hyperbilirubinemia and increased bile acid levels in all dogs. Bilirubinuria was evident in all of the affected dogs. In severely affected dogs, prolongation of bleeding times, thrombocytopenia and hypoalbuminemia were also present.

Gross necropsy findings included emaciation, jaundice, and yellow serous fluid in the peritoneal cavity. The liver was slightly enlarged, with numerous 2-3 mm hemorrhagic nodules, and occasional 1-2 cm firm nodules throughout. On histopathology, there was very severe acute and subacute periacinar and periportal necrosis with massive panlobular hemorrhage and necrosis in some lobules and moderate to extensive bile duct proliferation.

Toxicologic analysis (feed and tissues) for aflatoxins, iron, copper and zinc was essentially negative. The list of feed-related hepatotoxicants in dogs is short, and includes aflatoxin, iron, and zinc, as well as microcystin from blue-green algae. Pyrrolizidine alkaloid toxicity has not been reported in dogs. Feed analysis for aflatoxins would likely be negative unless we were fortunate to test the affected portion of the batch of feed.

Aflatoxin is a group of closely related chemicals produced as by-products of *Aspergillus flavus* and *A. parasiticus* commonly associated with corn, peanuts, and cottonseed meal, which can become infected during growth, transport or storage. Hepatotoxicity is the main syndrome, but carcinogenesis and immunotoxicity also occur in some species. Detection of the toxin in tissues or feeds can be extremely difficult or impossible at the time of onset of clinical signs and there is no specific treatment or antidote. Liver function may gradually return to normal in affected animals, but long-term effects are essentially unknown.

Reference

Roeder JD. Veterinary Toxicology. Butterworth-Heinemann. 2001: 72-74.

Contact bhoff@lsd.uoguelph.ca

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Johnston WT, Dewey CE, Friendship RM, **Smart N, McEwen BJ, Stalker M**, de Lange CFM. An investigation of the etiology of a mild diarrhea observed in a group of grower/finisher pigs. Can Vet J 2001; 42: 33-37.

Karasin AI, Olsen CW, Brown IH, **Carman S, Stalker M, Josephson G**. H4N6 influenza virus isolated from pigs in Ontario. Can Vet J 2000; 41: 938-939.

Lynn DH, Gransden SG, Wright ADG, **Josephson G**. Characterization of a new species of the ciliate *Tetrahymena* (Ciliophora: Oligohymenophorea) isolated from the urine of a dog: First report of *Tetrahymena* from a mammal. Acta Protozoologica 2000; 39: 289-294.

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Weese JS, Baird JD, Poppe C, **Archambault M**. Emergence of *Salmonella typhimurium* definitive type 104 (DT104) as an important cause of salmonellosis in horses in Ontario. Can Vet J 2001; 42: 788-792.

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Hobson J, Duffield T, Peregrine A, **McEwen B,** Hietala S, Lissemore K, Leslie K, Kelton D, Cramer G. Risk factors for *Neospora caninum* infection and associated abortion in Ontario Holstein dairy herds. Proc 34th Ann Conf Am Assoc Bov Pract, Vancouver, BC, Sept 13-15, 2001: 179.

McEwen B. Disease trends in eastern Ontario. Central Canada Vet Assoc, Kemptville, Ontario, June 12, 2001.

McEwen B, Mann E. Animal health and food safety surveillance for human health benefit. Symposium on Zoonotic & Communicable diseases. British Columbia Centres for Disease Control, Vancouver, British Columbia, April 2, 2001.

Measures L, **Parker L, McRaild P**, Hammill MO, Albert E. Oral mycoplasmal infections in pinnipeds - risk of "seal finger" infections in humans? Soc Marine Mammalogy Mtg, Vancouver, Nov 29, 2001. (poster)

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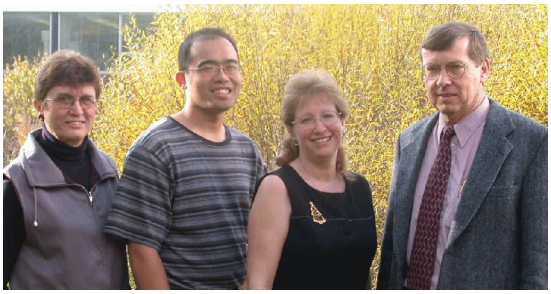
van Dreumel T. A review of the pathology of *Mycoplasma bovis*. Western Conf Diagn Vet Pathologists, Saskatoon, Oct 28, 2001.

Watson P. Understanding and using diagnostic labs. Sheep Production and Management Series, Kemptville, Jan 19, 2001.

AHL People

Molecular biology staff - from left to right, **Leah Read, Dr. Hugh Cai, Bhaju Tamot**. As you will have noted in this and previous issues of the AHL Newsletter, this group has been very busy with development of new DNA-based testing. To date, the AHL has added PCR tests for PRRSV, BVDV, porcine circovirus 2, *Leptospira spp., Streptococcus equi, Clostridium perfringens* serotyping, *Escherichia coli* genotyping. We are currently validating PCR tests for *Ehrlichia risticii, Salmonella spp., Brachyspira, Mycoplasma hyopneumoniae*, transmissible gastroenteritis virus, swine influenza virus, infectious bursal disease virus, and VTEC/STEC genotyping.



AHL administration - from left to right, **Mary Halfpenny** and **Michael Villagonzalo**, information technology; **Helen Oliver**, administrative assistant; **Dr. Grant Maxie**, manager. Absent: **Leslee Levy**, human resources representative.

AHL-Kemptville lab staff - front, **Alan Darch**, client service representative/post-mortem attendant; rear, left to right, **Dr. Jan Shapiro**, veterinary pathologist/lab outreach; **Dr. Phil Watson**, veterinarian; **Tom McLean**, client service representative/post-mortem attendant. We serve clients in eastern Ontario via the AHL- Kemptville lab, with support from AHL-Guelph. Necropsy services are performed by Dr. Shapiro, or by Dr. Watson in her absence. Fresh and formalinized samples are forwarded to Guelph for further testing. Test results are entered into VADDS as soon as they are verified in either Kemptville or Guelph, to form a cumulative report.