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Laboratory Services Division

Animal Health Laboratory



AHL Newsletter

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Companion animals Extraintestinal toxoplasmosis in cats

Our new AHL Animal Health Network Coordinator, Dr. Melanie Barham

On July 2, 2014, Dr. Barham joined the ranks of the AHL as our Animal Health Network Coordinator, under DSP, the Disease Surveillance Plan. Melanie will be our key contact as we organize our various species expert networks. Background on the DSP can be found at <u>http://www.uoguelph.ca/omafra_partnership/en/</u> <u>partnershipprograms/DiseaseSurveillancePlanDSP.asp</u> Plans are well advanced on the small ruminant expert network, with others to follow soon.

Melanie is a 2007 OVC DVM graduate with private practice experience in California, eastern Ontario, and locally, primarily as an equine practitioner. She also worked as a student at AHL-Kemptville. Melanie is based in the Pathobiology-AHL building on the Guelph campus, room 1816, and can be reached at barhamm@uoguelph.ca, phone 519-824-4120, ext 53364.

Welcome Melanie! *AHL*



Renovations! AHL-Guelph Specimen Reception will be undergoing renovation this fall to improve both our biosecurity and our service efficiency. We will do everything possible to minimize inconvenience to our clients. Writing sample IDs on your submission forms or sending Excel spreadsheets to <u>specroom@uoguelph.ca</u> will help us serve you faster.

Free specimen shipping to the AHL from within Ontario! Incoming Purolator collect account number 0966901

Specimen receipt confirmation

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The AHL now offers a "specimen receipt confirmation" email or fax. As soon as your submission is entered into LabVantage - our laboratory information management system - it generates an automatic report that is emailed and/or faxed to you indicating the specimens received, the tests that have been ordered, as well as the expected turnaround time for each test.

Please email us at <u>ahlinfo@uoguelph.ca</u> if you would like this feature activated.

Once activated, if you should happen to have questions or concerns about data entry or there are changes needed - please email <u>ahlinfo@uoguelph.ca</u> with the case number and concern and we will investigate.

UNIVER	SITY				
FGUEL	Рн			Case	# 14-053286
LABORATORY SERVICES				Submission Dat	
			s	Specimen Receipt Confirmation	
Submitter Infor	mation		Invoice Informati	ion	
	ne: MRS PHYLLIS FE	w	Clinic ID:	1773675	
	ID: 9999999			LABORATORY SERVICES DIVISOR	
Addres	IAB SERVICES T LABORATORY SE ADDRESS 1	ERVICES	Address :	LABORATORY SERVICES DIVISO 95 STONE RD W GUELPH, ON N1H 8J7	N
	CITY, ON POSTC CANADA	ODE		CANADA	
Pho	ne: -0113130123456	5	Phone:	519 823-1268	
	ax: 519 767-6240			519 767-6240	
Em	ail: pfew@uoguelph.c	a	Email:	pfew@uoguelph.ca	
Client Information	ABORATORY SERVI	CER DIVISION	Animal ID ·Example		d :16-Jul-2014
PO# 1		CES DIVISON	Species :Canine		a :10-Jul-2014 nt :16-Jul-2014
Quotation # :					d :17-Jul-2014
Insurance : 1			Sex :Male	Lega	II :N/A
Report Distributio	n				
LAB SERVICES T	EST ACCOUNT	Fax: N	Em	ail: you@uoguelph.ca	Mail: N
History	-				
"Open sore on left	hind leg."				
Specimens receiv	ed				
1 whole blood, 1 e	dta blood, slides				
Lab Sample ID	Client Sample ID	Date Sampled	Specimen Type	Test	Published TA (working days
Lab Sample ID			Blood - EDTA	CBC, companion-other	1.4
14-053286-0001	specimen 1		BIOOD - EDTA	CBC, companion-outer	10

AHL accreditation activities Grant Maxie, Nadine Ryan, Liz King

The AHL is accredited for testing by several different bodies, with continuing accreditation supported by periodic site visits and intensive audits.

As part of the **Standards Council of Canada** (SCC) audit in the fall of 2013, the AHL applied for and has now been approved under the category of "**flexible scope**" within our ISO/IEC 17025 registration. Rather than accrediting every test analyte in every matrix, we are now able to respond more quickly to client requests for accredited tests by doing our usual validation process for individual tests within the umbrellas of flexible scope for test technologies, namely

- 1. Culture detection of microorganisms
- 2. Inorganic analysis by inductively coupled plasma spectroscopy (ICP)
- 3. Enzyme-linked immunosorbent assay (ELISA)
- 4. Agglutination
- 5. Polymerase chain reaction

We are also accredited by SCC for "*Test method development & evaluation and non-routine testing*". http://palcan.scc.ca/specs/pdf/826_e.pdf

In June, 2014, AHL-Guelph and AHL-Kemptville were audited by 4 assessors representing the Accreditation Committee of the **American Association of Veterinary Laboratory Diagnosticians** (AAVLD), and we eagerly await their final report. The AHL has been fully accredited by the AAVLD, for all species, since 1993.

Given the comprehensive coverage of animals within the new **Ontario Animal Health Act, 2009** (AHA), the AHL has expanded into testing of **aquaculture fish** and **honey bees**. We are currently validating a wide range of tests for fish and bees, will accredit these tests as appropriate, and will soon be offering these tests to the fish and bee commodity sectors.

Automated reporting of positive tests for any of the **118 immediately notifiable hazards** in Ontario under the AHA has been in place since January 1, 2013, and is running smoothly. *AHL*

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Summary of bovine viral diarrhea virus (BVDV) testing at the AHL

CHANGING LIVES IMPROVING LIFE

AHL LabNote

Number 1

Updated by Davor Ojkic, DVM, PhD

TEST	SAMPLE TYPE	PURPOSE
PCR test for virus detection	EDTA blood, serum,	Primary test for both persistently and acutely infected animals – sensitive, rapid,
	plasma, tissues, ear notches, milk	cost-effective.
Herd screening: Test pools of 5-20	,	al samples so if needed lab can "open" pools to determine which animals were posi-
tive.	-	
		e infection: A positive PCR result on a single sample cannot distinguish between an
Acutely infected animal and a PI anii Acutely infected animals that have r		als at least 3 weeks after first testing. PI animals will be virus-positive a second time.
		f age, but some may survive longer. Bulk tank milk samples can be used to identify
		L of milk from a well-stirred bulk tank. The sample should represent a pool of not
more than 400 animals. Keep milk c		
If the test is negative, then individua		
If the test is positive, milk should be		
If the bulk milk sample is positive a		
		initially test first-lactation animals, removing any positives, followed by testing an- 3rd lactation animals that contributed to the bulk milk sample do not need to be test-
		imple, milk the remaining half of the herd and take a second sample. If only the sec-
		that contributed to the second milk sample.
TEST	SAMPLE TYPE	PURPOSE
Virus neutralization (VN) test	Serum	Cattle infected with a type 1 virus will have comparatively higher titers in a VN
for antibody detection		assay with BVDV type 1 (NADL). Similarly, cattle infected with a type 2 virus will
		have higher titers in BVDV type2 (NVSL) VN assay. For alpacas BVDV Type 1b
		TGAC will be used.
		erd has previously been exposed to BVDV.
		In naive animals, it may take as long as 4 weeks to develop a significant antibody lay for direct comparisons to be made, please submit the acute and convalescent sera
together.		
		ody by 6 to 8 months of age - if animals between the ages of 8 and 12 months have
		sposed to a field virus within the last few months. If they have been vaccinated with
		mmonly be less than 256. This strategy is not useful if young animals have been
vaccinated with a modified live viru		prior to ingestion of colostrum can be useful to demonstrate in utero infection and
can also be used to indicate "recent"		
Other tests available to detect viru		millional daily liolas.
TEST	SAMPLE TYPE	PURPOSE
Antigen ELISA	Ear notches, serum	To detect/identify persistently infected animals. Not recommended for acutely
	,	infected animals. For serum testing collect from precolostral calves or calves older
		than 3 months.
Virus isolation	EDTA blood, serum,	To detect both acutely affected and PI animals and determine if the virus is cyto-
	tissues	pathic (mucosal disease) or noncytopathic. However, virus isolation can take 2
		weeks.
Immunohistochemistry	Fixed tissue samples	When fresh tissues are not available, or as a part of postmortem procedures for indi-
		vidual diagnostics. Cannot be used for PI screening

This LabNote is available on our website at:

http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote01BVDV%20testingAprilFinal.pdf

Updated April, 2014

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AHL Lab Reports RUMINANTS

Listeriosis diagnoses in ruminants - AHL submissions, 2009-2014

Jan Shapiro, Beverly McEwen, Josepha DeLay

Listeriosis is a sporadic bacterial infection of ruminants almost always caused by *Listeria monocytogenes*, with 3 main clinical syndromes: abortion and perinatal mortality, septicemia of neonates, and neurological disease associated with encephalitis or meningoencephalitis of older ruminants. Between June 2009 and June 2014, listeriosis was diagnosed in 76 submissions to the AHL, Guelph and Kemptville locations. (Table 1). Listeria abortion was diagnosed in 8 cases, involving 6 cattle, 1 sheep and 1 goat submission. Neonatal *Listeria* septicemia was diagnosed in 3 cases, involving 1 bovid, 1 sheep and 1 goat submission. The majority of listeriosis cases were neurological, and consisted of 65 cases, of which 24 were sheep, 20 were goats and 21 were cattle.

Confirmation of a diagnosis of listeriosis has usually done by histology and bacterial culture. Positive identification of the agent is particularly important for diagnosis of *Listeria* abortion or septicemia, as **the history and gross and histopathology are not etiologically specific**. For some cases of encephalitic listeriosis, when the morphology and distribution of the histopathology fits the classical description for the disease, or when fresh tissue is not available for culture, the diagnosis may be based on histopathology alone.

In the March 2014 AHL Newsletter, we reported the validation of an immunohistochemical (IHC) test for L. monocytogenes using formalin-fixed, paraffin-embedded tissue sections, providing an important ancillary test in encephalitis cases for which brain histopathology is not classical for listeriosis, or fresh tissue is not available for culture. Another advantage of IHC is that, in cases in which a zoonotic viral agent is a rule-out, IHC can be safely performed without the need for handling fresh, potentially contagious, tissue. IHC may also be a useful test for cases in which histologic lesions are suspicious but bacterial culture results are negative for various reasons, such as contamination, autolysis or sample selection. In 2 recent cases of encephalitic listeriosis in goats for which brain histopathology was typical, the IHC test on fixed brain was positive, whereas bacterial culture of fresh brain was negative. AHL

D' ('	Sheep			Goats			Cattle		
Diagnostic tests	Abortion	Septicemia	Neurologic	Abortion	Septicemia	Neurologic	Abortion	Septicemia	Neurologic
Histology alone			14			6			11
Histology and bacte- rial cul- ture	1	1	10	1	1	8*	5	1	5
Bacterial culture						1			
Histology and IHC on brain						3			5**
Histology, bacterial culture and IHC on brain						2***			

Table1. Summary of the tests used for diagnosis of listeriosis cases, 2009-2014.

*In 2 neurological cases in goats, histopathology was fully consistent with listeriosis but bacterial culture was negative.

** In 1 bovine neurological case, histopathology was fully consistent with listeriosis but IHC was negative.

***In 2 goat neurological cases, histopathology was fully consistent with listeriosis, bacterial culture was negative, but IHC was positive. SWINE

AHL LabNote Number 30

July 24, 2014 ahlinfo@uoguelph.ca 519-824-4120 ext 54530

Undifferentiated diarrhea in pigs, including PEDV and PDCoV testing at the AHL

The AHL encourages veterinarians to submit samples from any cases of undifferentiated diarrhea. **We recommend that the triplex porcine coronavirus PCR for PEDV, TGEV, PDCoV (deltacoronavirus) be done** *BEFORE* **submitting animals to the lab for postmortem where diarrhea is a clinical sign - the triplex** PCR test will be done at no cost to clients as part of OMAFRA/AHL disease surveillance for the <u>initial</u> clinical diagnosis (Ontario submissions only).

Submissions for pigs with vomiting and diarrhea should include the samples listed below. **Take gut samples as soon as possible after euthanasia and place in formalin** to achieve optimal TGEV IHC test performance.

Tissue		Fresh	Formalin-fixed			
stomach		v	•			
duodenum			Ø			
jejunum		🔮 (X 2)	🔮 (X 3)			
ileum		•	✓ (X 3)			
cecum			⊘			
spiral colon		✓ (X 2)	•			
mesenteric LN		()	Ø			
other - liver, kid	ney, spleen, lung		•			
feces (leak-proo	f vial or swab),	0				
TESTS						
Histopathology			Routine histopathology IHC for TGEV, PCV-2			
Bacteriology	Colon or feces (NO swabs) for <i>Brachyspira</i> spp., <i>B. hyodysenteriae</i> , <i>B. pilosicoli</i> , <i>B. hampsonii</i> clade I and B. hampsonii clade II PCR.					
	Ileum or feces (NO swabs) for Lawsonia intracellularis PCR.					
	spp.) and anaerobic (C. pa	al tissue/content, feces, or fecal/intestinal swabs for aerobic (<i>E. coli</i> and <i>Salmonella</i> and anaerobic (<i>C. perfringens</i>) culture. or intestinal content (NO swabs) for <i>C. difficile</i> toxins ELISA.				
Virology	Live - fecal swab in VTM (preferred) or feces - rotavirus A/B/C PCR. Porcine coronavirus PCR (PEDV, TGEV, PDCoV).					
	Dead - PCV-2 PCR, rotation on frozen small intestine	virus A/B/C PCR, porcine or feces.	A/B/C PCR, porcine coronavirus PCR (PEDV, TGEV, PDCoV) eces.			
Serology	detect PEDV antibodies). PEDV ELISA and IFA ar quest. We await release o	e available at external lat	r differential TGEV/PRCV ELISA (does not poratories and the AHL can send sera on re- PEDV ELISA that can be offered at the			
Parasitology		AHL. Fecal exam optional (histology can be diagnostic).				

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HORSES

Eastern equine encephalitis in 2 horses in Ontario

Andrew Brooks

In August 2014, fatal eastern equine encephalitis virus (EEEV) infections were diagnosed in 2 horses in eastern Ontario.

The first case involved a **3 year old Canadian Sport Horse** that was presented with acute depression, ataxia, recumbency, drooling and fever. The clinical signs progressed and the horse was euthanized less than 24 hours after the onset. An IgM ELISA, performed at the National Veterinary Services Laboratory in Ames, Iowa, was positive for EEEV.

The second case involved a **12-year old Belgian gelding** that was presented with acute ataxia, nystagmus, strabismus, recumbency and fever. The horse became nonresponsive and was euthanized. An autopsy performed at the AHL-Kemptville revealed severe meningoencephalitis consistent with EEEV infection (Figure 1). The EEEV IgM ELI-SA was positive and EEEV was detected in the brain by real time RT-PCR at AHL-Guelph.

The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) was notified of the cases and a disease advisory was sent to the veterinary community.

Eastern equine encephalitis virus is a mosquitoborne pathogen capable of causing disease in horses, people, some avian species, as well as deer and dogs. EEEV is maintained in a cycle between mosquitoes and avian reservoir hosts. The virus is transmitted to horses by the bite of an infected mosquito. Horses (and humans) are considered dead -end hosts as they do not develop sufficient viremia to reinfect mosquitoes. EEEV affects mainly equine species in eastern North America and rarely may cause severe disease in humans. EEE is diagnosed only sporadically in horses in Ontario, with most **cases occurring in August and September**, although cases can occur later if the environmental conditions favor survival of mosquitoes.

The clinical signs in horses may include acute and progressive weakness, depression, fever, ataxia, seizures, and recumbency. The mortality rate in unvaccinated horses infected with EEEV is high; however, effective equine vaccines are available. The two horses in this report were unvaccinated.

Veterinarians in Ontario should consider EEE as a differential diagnosis in horses exhibiting acute neurological disease. The clinical signs are not specific for EEE and other differentials such as infection with West Nile virus, rabies virus, equine herpes virus 1. Botulism, hepatic encephalopathy, equine protozoal myeloencephalitis, tetanus and lead poisoning should also be considered.

In live horses, recent infection with EEEV is diagnosed with the **IgM ELISA performed on serum**. After autopsy, EEEV infection is confirmed by **real time RT-PCR**, immunohistochemistry, and characteristic histological lesions in the brain.

Please advise the laboratory if you are submitting a horse with suspected viral encephalitis - because of the zoonotic risk, such postmortems are conducted according to specific protocols. *AHL*

References

Equine Health Advisory, *Eastern Equine Encephalitis Confirmed in Eastern Ontario*. OMAFRA, August 11, 2014. http://www.cdc.gov/EasternEquineEncephalitis/

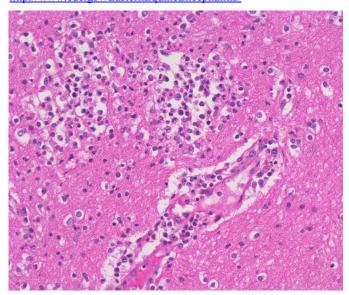


Figure 1. Severe neutrophilic and mononuclear cell inflammation in the cerebral cortex due to EEEV infection.

On-line access to your results? The AHL can provide easy confidential on-line access to client results through our LIMS. The URL for this is <u>http://</u><u>sapphire.lsd.uoguelph.ca:8080/labservices/logon.jsp</u> Please email <u>ahlinfo@uoguelph.ca</u> to sign up and receive your user name and password (this is an individual password different from the access to the Fee Schedule).

Remote entry of submissions? - Many of our frequent submitters are signed up for remote entry. If interested, please contact us at <u>ahlinfo@uoguelph.ca</u>

AVIAN/FUR/EXOTIC SPECIES

Chronic fowl cholera (*Pasteurella multocida*) in broiler breeder chickens *Emily Martin*

In March 2014, 10 live, 43-week-old broiler-breeder chickens were submitted to the AHL for postmortem examination. The presenting complaint was **twisted heads and necks**. These birds had been identified in the flock for the previous 2-3 weeks and there had been a sudden increase in the number of affected birds (up to 20) the day prior to submission to the AHL.

On examination prior to euthanasia, the majority of birds could not stand. Those that could stand had difficulty maintaining an upright position and their heads were down and twisting side to side (Figure 1).

On gross postmortem examination, it was noted that the frontal and parietal skull bones of most of the birds were discolored yellow and spongy (Figure 2). There were no other significant gross abnormalities and tissues were collected for histopathology and bacteriology.

On bacteriology, a pure culture of *Pasteurella multocida* (moderate to large numbers) was isolated from the cranial bones of 2 different birds.

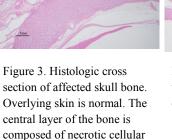
Pasteurella multocida is a contagious bacterial pathogen of domesticated and wild birds. It is a gram negative rod that can grow aerobically or anaerobically. It has variable pathogenicity that depends on the strain, variations within the strain, affected host species, variations within the host species, and the strain-host interaction. It usually causes septicemia with high morbidity and mortality, but chronic or benign conditions can occur. Turkeys are more susceptible than chickens and mature chickens are more susceptible than young. The bacterium usually enters through the mucous membranes of the pharynx, upper airways, conjunctiva, or cutaneous wounds. Some researchers suggest that the Eustachian tube could be a route of infection where the infection localizes in the air spaces of the cranial bones and middle ear.

On histopathology of the broiler breeder tissues, there were lesions of severe **osteomyelitis of the cranial bones** (Figure 3), **otitis media** (Figure 4), **meningitis** (Figure 5), **sinusitis and evidence of septicemia**. On close inspection of the ear sections, an area of bone necrosis and inflammation, including bacterial colonies, was identified between the air spaces of the skull and the meninges. Anatomically, the middle ear cavity is continuous anteriorly, posteriorly and dorsally with the pneumatized spaces of the avian skull. In cases of severe infection, there can be necrosis of the skull bone and extension of the infection into the meninges and adjacent brain tissue resulting in neurologic signs.

This case is consistent with a chronic, localized *Pasteurella multocida* infection. *AHL*

Figure 1. Broiler breeder with twisted head/neck.

Figure 2. Postmortem appearance of spongy yellow skull bones.



debris, heterophils and bacte-

ria (1X).

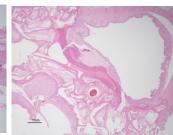


Figure 4. Histologic section of the avian ear containing necrotic debris (1X).

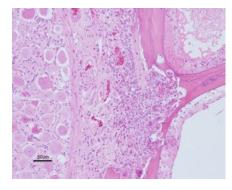


Figure 5. Histologic section of skull bone necrosis and inflammation of the adjacent meninges/brain tissue (20X).

COMPANION ANIMALS

Extraintestinal toxoplasmosis in cats Andrew Vince, Tamara Cohen, Beverly McEwen

Tissues were submitted to the AHL from a 22month-old outdoor domestic short haired cat that had been presented to the referring clinic for decreased appetite and lethargy of 1 week duration, with occasional bouts of vomiting. The cat was quiet, alert and responsive, febrile (39.9° C), thin and unthrifty. He was tender on abdominal palpation, but without localization of discomfort. In-clinic SNAP ELISAs for FIV, FeLV, and panleukopenia were negative. He was treated symptomatically over 3 days with IV fluids, antibiotics, appetite stimulants/antinauseants, and analgesics, but his fever failed to respond and actually rose in spite of therapy (40.5° C). On day 4 after presentation, the cat was agonal and was euthanized.

A postmortem was performed by the veterinarian; the liver was pale with an accentuated lobular pattern and mesenteric lymph nodes were enlarged (supporting the ultrasonographic findings), and a large quantity of yellow strawcolored fluid was in the thoracic cavity. There was widespread necrosis in both mesenteric lymph node and liver, with only marginal inflammation in the form of increased macrophages. Feline infectious peritonitis, toxoplasmosis and salmonellosis were initial differential diagnoses, but within macrophages in both liver and lymph node and within some hepatocytes were numerous regular 3-4 µm diameter round-elongate basophilic protozoa, typical of Toxoplasma sp. zoites. These organisms were positive with immunohistochemical stains for Toxoplasma gondii. Since 2008, 9 confirmed or strongly suspected cases have been recorded at the AHL in cats, typically between 0-2 cases/year, and are summarized in Table 1. In addition to these lesions, related lesions included 2 cases of adrenalitis, and 1 case each with enteritis, splenitis, and peritonitis.

Toxoplasma gondii has 3 developmental cycles: an **enteroepithelial cycle** (typical of definitive hosts, such as cats); the animal feeds on bradyzoite-infected tissues (often mouse brains), infecting jejunal enterocytes, which multiply through 6 phases and eventually discharges oocytes in the feces; an **extraintestinal cycle** (typical of non-definitive hosts), in which the animal feeds on infective oocysts followed by formation of rapidly-multiplying tachyzoites, which infect the lamina propria, mesenteric lymph nodes, and distant organs; and a **transplacental cycle** (similar to the extraintestinal cycle, with tachyzoite infection of fetuses). Cats tend to experience both intestinal and extraintestinal cycles after *Toxoplasma* infection, but often are subclinically infected unless immunosuppressed, as might occur with FIV or FeLV infection or with administration of drugs that modi-

fy immunity (glucocorticoids, cyclosporine, chemotherapeutics); however, there are reports of visceral toxoplasmosis in cats without any indication of immune compromise.

Most epidemiologic data on the prevalence of Toxoplasma gondii in animals and humans is based upon serologic data (indicating exposure but not necessarily active disease), and points to this being a common pathogen; however, clinical disease is uncommon in most domestic species except small ruminants, where it causes abortion. As a zoonotic risk, most publications indicate cats represent a low risk factor for human infection, with contamination of meat and vegetables a more likely infective source. Special precautions with respect to handling of feline feces (or soil that might be so contaminated) are usually reserved for those who are pregnant or immunosuppressed. Further details on the epidemiology of toxoplasmosis in animals and humans are available on the wormsandgerms blog (http:// www.wormsandgermsblog.com/M3%20Toxoplasma%20-% 20MD.pdf).

Toxoplasmosis should be considered a differential diagnosis for any fever of unknown origin or polysystemic illness in cats, for which histopathology and immunohistochemistry typically provides a definitive diagnosis. For diagnosis, recommended organs include: lung, liver, heart, brain, mesenteric lymph nodes, adrenal gland, small intestine, and spleen. *AHL*

Table 1. Case features for feline extraintestinal toxoplasmosis, AHL 2008-2014.

	History	Lesions
Total cases since 2008	9	
Average #/year	1.14	
Domestic shorthaired cats	8/9	
Domestic longhaired cats	1/9	
Male (castrated and uncastrated) cats	8/9	
Average/maximum age	2.3/9y	
History of fever/hyperthermia	4/9	
Respiratory disease	4/9	7/8
Hepatobiliary disease	3/9	7/9
Cardiovascular disease	3/9	4/8
Neurologic disease	2/9	5/7
Lymphadenitis/lymph node necrosis		5/8
Toxoplasma IHC definitive positive	7/8	
Toxoplasma IHC suspect positive	1/8	
FIP IHC positive	1/3	
Panleukopenia IHC positive	1/1	

AHL Newsletters and LabNotes are available on the Web at - http://ahl.uoguelph.ca