Laboratory Services Division
Animal Health Laboratory
University of Guelph
Changing Lives Improving Life

AHL Newsletter

Canada Post Publications number - 40064673

Volume 17, Number 1, page 1 **March, 2013** ISSN 1481-7179

In this issue:

EIA ELISAs are now run daily.

See P. 7 for details

Anaplasmosis cELISA accredited to ISO/IEC 17025:2005 at the AHL

Effective December 1, 2012, the CFIA changed its eradication policy to a **control policy**. In the case of a suspicious anaplasmosis test or suspicion of a clinical case of anaplasmosis, the CFIA will conduct confirmatory testing on the suspect animal. If the animal is confirmed positive, the herd will be quarantined, and all animals in the herd will be tested. All confirmed positive animals will be ordered to slaughter with compensation. After all cases have been sent to slaughter, the quarantine will be lifted.

Until April 1, 2014, the CFIA will not carry out testing of contiguous and traced herds, but will recommend to owners of these herds that they consider having their herds tested by a private veterinarian, with the samples submitted to a CFIA-approved laboratory, e.g., the AHL. **Effective April 1, 2014, anaplasmosis will be removed from the list of Reportable diseases and placed on the list of Immediately Notifiable diseases, and the CFIA will no longer respond to anaplasmosis reactors**.

The AHL *Anaplasma antibody competitive ELISA* is now officially accredited by the Standards Council of Canada (SCC) and the new scope of testing is posted on the SCC website at http://palcan.scc.ca/specs/pdf/826_e.pdf

The test requires 0.5 mL of serum, and the testing fee is $5.75 per test. Tests will be batched. Please contact the AHL Virology lab at ahlvirology@uoguelph.ca *AHL*

Merger of the AHL virology labs

Further to the retirement of Dr. Susy Carman in December of 2012, we have merged the Avian Virology and Mammalian Virology labs into the **AHL Virology** lab under the leadership of **Dr. Davor Ojkic**, with the technical supervision of **Keith Harron**, and assistance of team leads **Elizabeth Hillyer** and **Joanna Sawicki**.

There are no immediate changes to the spectrum or scheduling of tests, and we are committed to continuing to expand our ISO/IEC 17025:2005 scope of accreditation.

To streamline communication with our large group, we have introduced a new lab email address "**ahl.virology@uoguelph.ca**" - **please email us for test changes and/or other requests**. If you need to reach us by phone, please call 519 824 4120, extension 54514. *AHL*

We're going green!!

We plan to print 2 more issues of the AHL Newsletter - March and June, 2013 - then we will migrate to electronic publication. The AHL Newsletter has been available on-line for several years, and is easily accessible at http://guelphlabservices.com/AHL/Newsletters.aspx

Notification and Table of Contents of each newsletter will be sent to all addresses currently on our email distribution list - click on any topic of interest. If your practice is on the list, but you would like to receive a **personal notification**, please send your request to: *holiver@uoguelph.ca*

We welcome your feedback.

Selected AHL outreach activities, 2012

Bell-Rogers P, Parker L, Rosenbusch RF, **Cai H**. Development of multi-locus sequence typing assay and analysis of *Mycoplasma bovis i*solated from Ontario, Canada in the past three decades. Cong Intrntl Mycoplasmol Org, July 15-20, 2012, Toulouse, France.

Brash M, Weisz A. Spontaneous ALV-like lymphoid leukosis in broiler breeders in Ontario. OAPP mtg. Guelph, ON. Feb 14, 2012

Brash M, Dam A. Biosecurity and pigeon diseases. South Western Pigeon Club, London, ON. Feb 25, 2012

Brash M. 1. Eastern poultry disease update. 2. Dr. McMillan case study II. Annual Poultry Service Industry Workshop, Banff, AB. Sept 25- 27, 2012.

Brash M. Poultry health update. Poultry Prducr Updts. Brodhagen, ON. Dec 12, 2012.

Brash M, MacAlpine M, DeLay J, Ojkic D. Outbreak of Eastern equine encephalitis in ring-necked pheasants in Ontario. Western Poultry Dis Conf, Scottsdale, AZ, April 2-4, 2012

Brash M. Ouckama R. Leg abnormalities in Ontario broilers. WestVet 23. Abbotsford, BC. Oct 2, 2012.

Brash M, Weisz A. Spontaneous ALV-like lymphoid leukosis in broiler breeders in Ontario. WestVet 23. Abbotsford, B.C. Oct 2, 2012.

Brooks AS. Diseases of sheep. Ontario Sheep Marketing Agency District 10 Sheep Day. Feb 18, 2012 Spencerville ON.

Cai H, Bell-Rogers P. Development of biotechnology assays for *Mycoplasma* ID. AHSI wrap-up program. Guelph, ON. Nov 8, 2012.

Cai H, Parker L, Caswell J. Antimicrobial resistance of *Mycoplasma bovis* isolated from 1978 to 2009 in Ontario. Congr Intrntl Mycoplasmol Org, July 15-20, 2012, Toulouse, France.

Carman S, McEwen B: How does the AHL develop and validate new tests? AHSI wrap-up program, Nov 8, 2012

Carman S, McEwen B, DeLay J, Hazlett M, Stalker M. *Bovine viral diarrhea virus* (BVDV) testing at the AHL, U of Guelph, Ontario, Canada 1989 to 2011. OMAFRA Animal Health Forum, Guelph, ON, Mar 8, 2012.

DeLay J, Lapos S, Hazlett M. Development of an immunohistochemical test for small ruminant lentiviruses (CAEV, MVV). AHSI wrap-up, Nov 8, 2012, Guelph, ON.

Hazlett M, McDowall R, DeLay J, Stalker M, McEwen B, van Dreumel T, Spinato M, Binnington B, Slavic D, Carman S, Cai H. Investigation of infectious etiologies of small ruminant abortion in Ontario with emphasis on *Chlamydophila abortus* and *Coxiella burnetii*. AHSI Wrap-up Session, Nov 8, 2012, Guelph, ON.

Martin E. The AHL and testing available for fur producers. Ontario Fur Breeders Assoc Ann Mtg. Niagara Falls, ON, April 2, 2012.

Martin E. Mink necropsy demonstration. Ont Fur Breeders Assoc Fall Field Day. North Am Fur Auction, Toronto, ON. Oct 17, 2012.

Martin E, Brash M. Causes of elevated early mortality in poultry. CFIA Hatchery Inspector Training Lecture. Guelph, ON, Oct 4, 2012.

Maxie G. Animal Health Laboratory and AHSI overview. AHSI wrap-up program. Guelph, ON. Nov 8, 2012.

Maxie G. 2011-12 Ontario pathology update. CAVP. Winnipeg, MB, June 3, 2012.

Maxie G. Disease testing and analysis. CFIA VetProfUpdate. GuelphON. May 9 2012.

Maxie G. AHL update. OMAFRA Animal Health Forum. Guelph. March 8, 2012.

Maxie G, Fairles J. Animal Health Laboratory update and tour. CFIA Guelph District Office staff. Guelph, ON. March 20, 2012.

Maxie G, Hazlett M, Cai H, Fairles J, McEwen B. Animal Health Laboratory update. Ont Assoc Swine Vets. Guelph, ON. Oct 12, 2012.

McEwen B, Brash M, DeLay J, Hazlett M, Martin E, Spinato M, Stalker M. Forensic pathology at a diagnostic laboratory – a pathologist's perspective. IVFSA Ann Mtg, Miami, FL. May 2012.

McEwen B, Stalker M, DeLay J, Hazlett M, Martin E, Brash M, Spinato M, van Dreumel T. Animal Health Laboratory & OSPCA investigations. OSPCA Investigators, Newmarket, ON. Feb 8, 2012.

McEwen BJ, Stalker M, DeLay J, Hazlett M, Martin E, Brash M, Spinato M, van Dreumel T. Forensic pathology at the AHL. I. The basics, II. Post-mortem, neglect and non-accidental injury. III. Dog-fighting, sexual abuse, expert witness. Students CVMA Ann Mtg, Guelph, ON, Jan 13, 2012.

Nham E, Guerin M, Pearl D, **Slavic D, Ojkic D**. Prevalence and geographical distribution of avian reovirus in Ontario broiler chicken populations. IBH Industry Broiler Health Group Mtg, May 25, 2012; Guelph, ON.

Kasab-Bachi H, Guerin M, McEwen S, Pearl D, **Slavic D**, Boecker A. Prevalence, genotypes, distribution, seasonal patterns of *Clostridium perfringens* in broilers. IBH Industry Broiler Health Group mtg, May 25, 2012; Guelph, ON.

Nham E,Guerin M, **Ojkic D**, Pearl D, **Slavic D**. Epidemiological study of avian reovirus in Ontario broiler chickens. 2012 Inaugural Master's Level Grad Res Conf, April 14, 2012; Brockport, NY, USA

Nilsson S, Guerin M, Medhanie G, **Slavic D**. Speciation of *Brachyspira* spp. in poultry fecal samples using the GS Junior. AAVLD ann mtg, Greensboro, NC. Oct, 2012.

Ojha S, **Cai H, Hazlett M,** Keown B, Chen S. Molecular typing of *Coxiella burnetii* identified in Ontario by multiple-locus variable number of tandem repeat analysis (MLVA), AHSI Wrap-up Session, Nov 8, 2012, Guelph, ON.

Ojkic D, Martin EAK, Brash M. Infectious bronchitis virus update. OAPP meeting, Guelph, ON, Nov 1, 2012.

Slavic D. Clinical microbiology in the 21st century. How far did we get? Mike Wilson Swine Day. May 22, 2012.

Slavic D, Fairles J. Mastitis workshop for veterinarians. Guelph ON. Apr 23, 2012.

Spinato MT, Farzan V, McLeod KGW. Emergency preparedness status of Canadian veterinary diagnostic laboratories. OMAFRA/U of Guelph Emergency Mgmt Research Expo, Guelph, ON. Dec 7, 2012.

Spinato M, Shapiro J. Lessons learned from conducting FAD emergency exercises in the veterinary necropsy laboratory. CAHLN. Winnipeg, MB. June 4-6, 2012.

Spinato M, Shapiro J, Stalker M, Brooks A, Hazlett M. Emergency preparedness in the necropsy laboratory. AHSI wrap-up session. Nov 8, 2012, Guelph, ON.

Sunohara-Neilson J, **Brash M, Carman S,** Nagy E, Turner PV. Experimental infection of SPF New Zealand white rabbits with *Leporid herpesvirus 4*. AALAS Ntnl Mtg, Minneapolis, MN, Nov 4-8, 2012.

AHL Newsletter
March, 2013 - Volume 17, Number 1

Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistants: **Helen Oliver, April Nejedly**

The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends. *Ideas and opinions expressed herein do not necessarily reflect the opinions of the University or the Editor.*

Articles may be reprinted with the permission of the editor and with appropriate credit given to the AHL Newsletter.

Mailing address & contact information:
(**please return all undeliverable Canadian addresses to**:)
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
Email: holiver@uoguelph.ca

ISSN 1481-7179
Canada Post Publications number - 40064673

Contributors to this issue:
From Laboratory Services / Animal Health Laboratory:
Marina Brash, DVM, DVSc, Diplomate ACVP
Andrew Brooks, DVM, PhD, Diplomate ACVP
Hugh Cai, DVM, MVSc, DVSc
Josepha DeLay, DVM, DVSc, Diplomate ACVP
Jim Fairles, DVM, MBA
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Brent Hoff, DVM, DVSc, DipTox
Emily Martin, DVM, MSc, Diplomate ACPV
Beverly McEwen, DVM, DVSc, Diplomate ACVP
Davor Ojkic, DVM, PhD
Kristiina Ruotsalo, DVM, DVSc, Diplomate ACVP
Nick Schrier, MSc
Jan Shapiro, DVM, DipPath, DipEqSurg
Durda Slavic, DVM, PhD
Margaret Stalker, DVM, PhD, Diplomate ACVP
Other contributors:
Eileen deVilla MD, FRCPC, MOHTLC, ON
Nicola Mercer MD, FRCPC MOHTLC, ON
Andrew Peregrine, BVM, PhD, DVM, Pathobiology, Ontario Veterinary College, Guelph, ON
Alicia Skelding BSc MSc, OVC 2013, Guelph ON
Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

Selected zoonotic pathogens and diseases identified at the AHL, 2012

Beverly McEwen, Durda Slavic, Davor Ojkic, Josepha DeLay, Hugh Cai, Margaret Stalker, Murray Hazlett, Andrew Brooks, Kristiina Ruotsalo, Brent Hoff

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 about 1,000 cases annually (Tables 1 and 2). These are numerator data reliant upon submission biases to the laboratory and cannot be regarded as population prevalence estimates. Monitoring programs are not included. As of this year, only cases originating from Ontario are included. The case of canine *Echinococcus multilocularis* is described in detail on page 8. *AHL*

Table 1. Cases with selected zoonotic pathogens isolated and/or identified at the AHL, 2012 (ND = not done)

Pathogen	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2012	2011	2010	2009	2008
Ascarids (*T. canis, T. cati, T. leonina, Baylisascaris* spp.)								21	11	3	**35**	ND	ND	ND	ND
Blastomyces dermatitidis								10			**10**	10	5	10	ND
Bordetella bronchiseptica		18	2					5		8	**33**	43	54	60	52
Borrelia burgdorferi (Lyme disease)								3			**3**	1			
Campylobacter coli / jejuni / fetus subsp. *fetus*				12				4		1	**17**	12	24	14	14
Chlamydophila abortus				12	21						**33**	39	58	29	10
Clostridium difficile	1	11	7								**19**	40	31	24	25
Coxiella burnetii	2			15	19						**36**	99	115	9	15
Cryptococcus spp.										1	**1**				
Cryptosporidium spp.	122	8		2	7		1			1	**141**	147	157	128	144
Eastern equine encephalitis virus											**0**	5	12	11	12
Echinococcus multilocularis								1			**1**	0	0	0	0
Giardia spp.	2				2			20	1	1	**26**	31	60	55	56
Listeria monocytogenes	8			8	1			1			**18**	18	19	18	14
Methicillin-resistant *Staphylococcus aureus* (MRSA)			23					1			**24**	49	74	36	51
Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)								112	2		**114**	192	ND	ND	ND
Rabies											**0**	0	3	8	4
Salmonella spp.	76	46	25	3	1	63	28	5	2	32	**281**	256	256	281	322
Streptococcus suis	12	125		6	1						**144**	106	110	120	158
Streptococcus equisimilis	1	23	10					9		2	**45**	59	48	43	76
Streptococcus zooepidemicus	1		3								**4**	149	152	117	108
Toxoplasma gondii				2	5					1	**8**	24	22	19	8
Verotoxigenic *E. coli*	6	5			1						**12**	ND	ND	ND	ND
West Nile virus			7							29	**36**	34	7	6	70
Yersinia enterocolitica	1	1									**2**	1	2	0	6

Table 2. *Leptospira spp*. seropositive cases identified at AHL, 2012, microscopic agglutination test (MAT)

Leptospira spp. serovar	Bovine	Swine	Equine	Canine	Other
L. autumnalis	2		1	2	1
L. bratislava	4	1	10	9	1
L. canicola	7		4	9	1
L. grippotyphosa	6	1	4	21	1
L. hardjo	5				1
L. icterohaemorrhagiae	14	1	9	14	1
L. pomona	18	1	7	21	1
Antigen or nucleic acid test positive				1	3

AHL Lab Reports

RUMINANTS

Isolation of *Salmonella* Dublin in bovine respiratory disease

Durda Slavic, Murray Hazlett

A 4- to 5-month-old Holstein bull calf showed signs of respiratory disease 3 weeks after arrival in a feedlot, and was submitted to AHL for postmortem examination. Severe cranioventral suppurative bronchiolitis and alveolitis with large foci of necrosis involving 50% of both lung fields was detected. Affected lung was red, heavy, rubbery and mottled on cut surface. Microbiologic testing revealed the presence of numerous pathogens including *Bovine respiratory syncytial virus* (BRSV), *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Histophilus somni*, *Mycoplasma bovis*, and *Salmonella enterica* subsp. *enterica* serovar Dublin (*S.* Dublin).

***S.* Dublin is a host-adapted *Salmonella* of cattle.** It can affect cattle of all ages, but it is usually more severe in younger, immunologically naive animals. Calves can be infected at calving but do not necessarily show any clinical signs until they are 2-3 weeks old. In general, disease caused by *S.* Dublin is most frequently reported in cattle over 3 months of age. Clinically, calves may become anorexic with fever and diarrhea with or without the presence of blood. More frequently, however, *S.* Dublin in calves will cause septicemia and/or pneumonia in the absence of any enteric clinical signs. In adult animals, *S.* Dublin disease is clinically milder, but diarrhea, abortions, and respiratory disease have been reported. Affected adults become carriers and a potential source of infection to the rest of the herd by actively shedding the bacteria for months after the initial infection.

Although *S.* Dublin has been causing problems in cattle in the northeastern United States for years now, it has not been previously reported in Ontario. It is likely that this organism is not a primary pathogen in our case considering the large numbers of other pathogens identified, but our findings indicate that ***S.* Dublin should be added as a differential diagnosis not only for enteric disease of cattle but more importantly for cases of septicemia and respiratory problems**. Other non-Ontario cases have been seen recently.

If *S.* Dublin is suspected, a piece of lung or fecal material are the preferred samples rather than swabs. *AHL*

Reference

McDonough PL, et al. *Salmonella enterica* serotype Dublin infection: an emerging infectious disease for the Northeastern United States. J Clin Microbiol 1999;37:2418-2427.

Transfer of AHL mastitis results into DHI/DairyComp 305

Jim Fairles

The AHL, with collaboration and thanks to CanWest DHI, can now offer uploading of AHL milk bacteriology data to DairyComp 305 cow files.

We welcome this in order for veterinarians to have one more tool in dairy health management consulting. The DairyComp staff have produced documentation for looking at this data in DairyComp, and this will be available soon.

To facilitate this change, there is a new AHL mastitis submission form that can be used for any milk bacteriology submission. It can be found at http://www.guelphlabservices.com/files/Mastitis%20DHI%20form%20V03%20Feb%202013.pdf Please use this form and destroy all old forms. If you are using customized forms - please contact us at ahlinfo@uoguelph.ca and we will gladly provide customized milk bacteriology forms with clinic information.

Information that is mandatory for uploading of this AHL data to DHI is:

1. Your **client's DHI number** (8 digit number beginning in 050) - there is a new field on the form for this information under the Owner Unique ID.
2. The **exact cow ID** as it is listed in DairyComp. To facilitate the reduction in errors, there may be a slight delay in the data going to the "loop" to take into account field rep visits to add new cows etc. You will still receive the AHL results as usual when the case is complete.
3. **Sampling date**.
4. **Sample ID** - LF, LH, RF, RH, C, or BT (bulk tank). Please note, if there is an incomplete composite sample, e.g., LF LH - it will be coded as C (composite).

We thank all IT staff from AHL and CanWest DHI for making this change possible, and we look forward to implementation. *AHL*

SWINE

A retrospective look at transmissible gastroenteritis (TGE) diagnosis1988-Jan 2013 and optimizing diagnosis of piglet diarrhea

Beverly McEwen, Josepha DeLay, Davor Ojkic, Jim Fairles

In recent years, TGE has been a sporadic diagnosis at the AHL, but a confirmed case of TGE in January 2013 at the AHL prompted us to examine the number of diagnoses since 1988. The absolute number and proportion of TGE cases positive (by FA and/or IHC) have decreased substantially since 1998, and there were no cases from 2010-2012 (Figure 1). Cases occur most frequently during the winter months (Figure 2).

Testing for TGEV and other causes of neonatal diarrhea requires that intestinal mucosa is intact and well-preserved. Samples may be collected from on-farm postmortem of euthanized piglets, or live piglets may be submitted to the AHL for euthanasia and postmortem. Choose 3 recently affected, untreated pigs for postmortem in order to optimize lesions and antigen load, and ensure the most useful test results.

For on-farm postmortem, euthanize and postmortem piglets individually (i.e., do NOT euthanize all piglets at once, or epithelial preservation will be compromised). Immediately after euthanizing the pig and opening the carcass, collect intestinal samples – this variation from the standard order of events is known as a ***GI-targeted postmortem***. **Histology samples should have top priority** – collect samples at 2 sites from each of duodenum, jejunum, ileum, and colon, and place immediately in formalin. 'Swishing' each sample in formalin as it is placed in the container helps to ensure that mucosa is exposed to formalin, and is therefore well preserved. These samples will be used for both routine histopathology and for TGEV IHC.

To rule out other causes of neonatal diarrhea, submit a fresh section of jejunum for **porcine rotavirus A/C and rotavirus B PCR**, and a fresh section each of ileum and colon for **bacterial culture**. Samples from all 3 piglets may be pooled for each PCR test, making the tests more economical. *AHL*

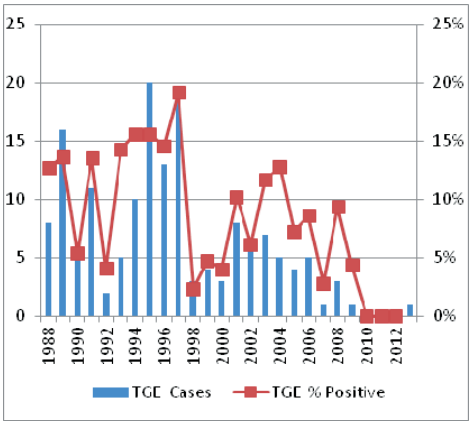

Figure 1. Number and percent confirmed positive TGE cases, AHL, 1988-Jan 2013.

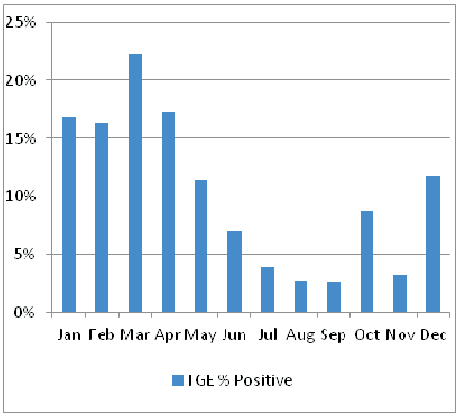

Figure 2. Proportion of TGE test positive cases, by month, AHL, 1988-Jan 2013.

Swine dysentery update, Ontario, 2013 *Durda Slavic*

Two cases of swine dysentery were diagnosed by the AHL in January 2013. Both cases originated from grower-finisher pigs and clinically were characterized by the presence of mucohemorrhagic diarrhea. While our first case originated from an antimicrobial-free herd, no information about antimicrobial status was provided for the second herd. *Brachyspira hyodysenteriae* was confirmed by both culture and PCR as the causative agent of dysentery in our first case. In our second case, PCR also confirmed the presence of *B. hyodysenteriae,* whereas culture is still ongoing. Histological lesions characteristic of swine dysentery were present in both cases. No novel *Brachyspira* spp. were detected.

If you suspect swine dysentery, submit feces or tissue samples to the AHL for *Brachyspira* culture and PCR. Although AHL PCR methods that will detect novel *Brachyspira* spp. clade I and clade II isolates are not fully validated, they are available and any positive sample will be sent to an independent laboratory for confirmation. Call the AHL laboratory in advance to alert us that samples are coming, since a selective medium for culture is made on an as-needed basis. *AHL*

AVIAN/FUR/EXOTIC SPECIES

Gallibacterium anatis - a review of culture-positive cases from commercial poultry submitted to the AHL in 2011 and 2012

Jan Shapiro, Marina Brash, Emily Martin, Andrew Brooks, Durda Slavic, Beverly McEwen

A recent report of an increase in the prevalence of isolates of the bacterium *Gallibacterium anatis* (formerly belonging to *Pasteurella anatis-Pasteurella haemolytica* complex) in chickens from the southern US prompted us to do a review of cases submitted to the Animal Health Laboratory from commercial poultry operations in 2011 and 2012. The purpose was to determine from which tissues *G. anatis* was cultured, and which, if any, disease conditions were associated with a positive culture. *G. anatis* was cultured from 5 cases in 2011 and 10 cases in 2012. Positive cases included 1 submission of immature broiler breeders, 5 submissions of broiler breeders in production, 5 submissions of broiler chickens, and 4 submissions of laying hens.

In young **broiler breeder** males, *G. anatis* was isolated from pooled livers in birds with mixed bacterial septicemia. In broiler breeders in production, it was cultured with *Staphylococcus aureus* from the shoulder joint of birds with arthritis, from pooled livers in birds with mixed bacterial septicemia and arthritis, in pure culture or with *E. coli* from liver and spleen pools of birds with septicemia, from air sacs of birds that also had *E. coli* fibrinosuppurative pneumonia and pleuritis, and in mixed culture with *E. coli* from wattles of birds with fowl pox.

In **broiler chickens**, *G. anatis* was cultured from multiple tissues including bone, lungs, joints, pericardium and pools of livers and spleens. It was always isolated in mixed culture with *E. coli*, and from the joint swab, it was also isolated with *Enterococcus cecorum* and *Salmonella* Heidelberg. In the broiler chicken cases, a detailed flock history was not provided and only fresh tissue or swabs were submitted for culture. Hence, no interpretation of the significance of a positive culture for *G. anatis* can be made from these cases.

In **laying chickens,** *G. anatis* was isolated in pure culture or in mixed culture with *E. coli* from the oviduct of hens with salpingitis, and in mixed culture with *E. coli* from yolk of a hen with peritonitis.

Gallibacterium anatis has been considered to be part of the normal flora of the upper respiratory and lower genital tract of chickens. However, various authors have reported isolating it in pure culture from a range of lesions in poultry, including salpingitis, oophoritis, peritonitis, septicemia, pericarditis, hepatitis, enteritis and respiratory tract lesions. **In our submissions, *G. anatis* was isolated from cases diagnosed with septicemia, arthritis, osteomyelitis, pneumonia, pleuritis, pericarditis, peritonitis, salpingitis and infection of the wattles, but only rarely was it isolated in pure culture. The most frequent co-isolate was *E. coli*.**

Gallibacterium anatis has been reported in pure culture and as a co-infection with *E. coli* from reproductive tracts of laying hens with salpingitis and drop in egg production. Under certain conditions, e.g., impaired host immunity, co-infections and environmental factors, *Gallibacterium* isolates residing in the upper respiratory or lower genital tract may gain access to the systemic circulation and/or the upper reproductive tract and cause disease. Both *E. coli* and *G. anatis* may spread as ascending infections from extensive pecking wounds around the cloaca.

An additional concern about *G. anatis* is the reported nearly complete resistance of isolates to multiple (4) antimicrobials. **Of the 10 AHL isolates for which antimicrobial susceptibility testing was done, 10 were resistant to tetracycline, 9 to erythromycin, 5 to penicillin G, 5 to sulfonamides, 4 to kanamycin, and 4 to trimethoprim/sulfa.** We will continue to monitor the prevalence, associated diagnoses and antimicrobial susceptibility of positive isolates of *G. anatis* in future submissions. *AHL*

The AHSI avian *Chlamydophila* and *Coxiella* project wraps up

Marina Brash, Hugh Cai, Emily Martin, Margaret Stalker

The AHL Animal Health Strategic Investment (AHSI) project examining the prevalence of *Chlamydophila psittaci*, *Coxiella burnetii* and the avian *Coxiella*-like bacterium in psittacine birds, and the utility of different diagnostic tools and sample types, ended on January 31, 2013.

Thank you to all the veterinarians who submitted psittacine birds to the AHL for postmortem since the inception of the project in March 2011, as they were all included in the study. Results and outcomes of this research project will appear in future newsletters. *AHL*

HORSES

Hyperammonemia and encephalopathy associated with inflammatory intestinal disease in 2 horses.

Margaret Stalker, Murray Hazlett, Nick Shrier, Brent Hoff

Encephalopathy associated with elevated blood ammonia levels is well-recognized in horses, and is typically associated with significantly reduced functional hepatic mass and decreased hepatic clearance of NH_4^+. However, **cases of hyperammonemia with no evidence of abnormal hepatic function have also been recognized in horses for nearly 20 years, often in association with intestinal disease.** Two recent cases from the AHL are described here.

The first was a **3-year old pony gelding** with sudden onset marked ataxia, head-pressing, abnormal mentation and apparent blindness. Response to symptomatic treatment with dexamethasone and banamine was poor, and the animal was euthanized and presented to the AHL for postmortem examination. Clinical rule-outs included viral encephalitis. Gross pathology findings included subcutaneous bruising about the head due to self trauma. Testing of brain for rabies and PCR testing for EHV-1, WNV and EEEV were all negative. Liver lead levels were undetectable. On histologic examination of the brain, no inflammatory lesions were found. Instead, numerous clusters of large swollen astrocytes with vesicular nuclei were present in the cerebral cortices. These reactive astrocytes (Alzheimer type II cells), are considered a hallmark of elevated blood ammonia levels. No significant gross or histologic changes were present in the liver, however, histology revealed inflammatory changes in the duodenum and proximal jejunum compatible with acute proximal enteritis. Aqueous humor was analysed for NH_4^+ levels, and compared to control samples from 2 horses presented for postmortem for unrelated conditions. Aqueous humor NH_4^+ levels from the pony were 143 mg/L, whereas controls were both <1 mg/L.

The second case was a **2.5-year-old Thoroughbred colt** with a recent history of colitis, depression and pyrexia. The colt had been previously treated with ceftiofur for a respiratory infection, and was passing large volumes of watery diarrhea. After administration of fluid therapy and flunixin, the colt developed neurologic signs, with an abnormal hindlimb gait and profound depression. Clinical rule-outs at this time included neurologic EHV-1 with secondary colitis. Postmortem examination revealed ulcerative typhlocolitis with abundant watery yellow content. Cultures for *Salmonella* spp., and *C. difficile* toxin ELISAs were negative as was testing for rabies and EHV-1. Histology of the brain also revealed clusters of Alzheimer type-II astrocytes within sections of cerebral cortex. Aqueous humor NH_4^+ was 350 mg/L, again markedly elevated above levels in a control horse.

Hyperammonemia may be due to 1) increased production in the intestinal tract, 2) increased absorption due to increased intestinal permeability, or 3) decreased hepatic clearance. Of these 3 causes, **hepatic injury resulting in decreased ammonium clearance is certainly the most common cause of hyperammonemia and subsequent so-called "hepatic encephalopathy" in horses**. Typical clinical signs of hyperammonemic encephalopathy include depression, head pressing, ataxia, central blindness, circling, erratic behavior and recumbency. In a recently published case series of hyperammonemia in horses associated with primary intestinal disease of various causes, elevated blood ammonium levels sufficient to result in clinical signs of encephalopathy were thought to be associated with either increased gut permeability due to inflammatory intestinal disease, or a shift to urease-producing bacteria in the gut.

Hyperammonemia can be difficult to document on necropsy examination, however, analyses of aqueous humor or CSF collected at postmortem have been suggested as useful alternatives to plasma for diagnosing hyperammonemia in horses. *AHL*

Electronic reporting of EIAV results

The AHL now offers *Equine infectious anemia virus* antibody ELISA testing daily Monday through Friday, excluding statutory holidays. We provide 5 options for obtaining your EIA results:

1. **Canada Post mail** - standard (for original)
2. **Courier** - please indicate on the form, additional charge (for original)
3. **On-line access** to results (for convenience - no need to call the lab)
4. **Fax** (please indicate on form and include fax number)
5. **Email** (please indicate on the form and include email address if different than the email address we have on file)

To activate on-line access, please contact us at ahlinfo@uoguelph.ca Telephone: 519-824-4120 ext. 54530.

Once test results have been finalized, clients will continue to receive the standard AHL report indicating that the EIA test has been completed. This report now says "**Results to follow and available on-line**". The traditional AHL method for the return of original documents is by Canada Post mail. EIA original forms will be returned by courier when requested on the top of the CFIA EIA form - there will be an additional charge of $5.25 per courier package. *AHL*

COMPANION ANIMALS

Alveolar hydatid disease (*Echinococcus multilocularis*) in a dog from southern Ontario

Andrew Brooks, Alicia Skelding, Margaret Stalker, Nicola Mercer, Eileen deVilla, Andrew Peregrine

A 2-year-old, male, castrated Boxer dog was presented to a veterinary clinic in southern Ontario for anorexia, lethargy and reluctance to move. The dog exhibited tachycardia, tachypnea and abdominal pain. Radiographs revealed loss of serosal detail throughout the abdomen. Significant hemoabdomen was observed during exploratory laparotomy, with active bleeding originating from a large, tan, nodular, space-occupying ~10 cm diameter lesion in the liver (Figure 1). The liver lesion had eroded hepatic vessels resulting in uncontrollable hemorrhage. Given the poor prognosis, the owner elected euthanasia.

Histologically, the liver lesion was a multiloculated cystic structure composed of fragmented hyaline membranes, necrotic debris, mineralized granular material, and very severe eosinophilic granulomatous inflammation (Figure 2). The lesion was suspected to be an intermediate stage of a cestode parasite although protoscolices were not found in the lesion despite examination of numerous sections.

PCR amplification and sequencing, along with PAS staining, performed on formalin-fixed tissue at the Institut fur Parasitologie (Bern, Switzerland), confirmed that the liver lesion is an alveolar hydatid cyst (metacestode) of *Echinococcus multilocularis*.

***E. multilocularis* is a zoonotic tapeworm found in Canada, the United States and Europe**. In Canada, *E. multilocularis* is endemic in wildlife in the northern tundra zone of the Canadian arctic as well as the southern regions of Manitoba, Saskatchewan, and Alberta. Alveolar hydatid disease in a domestic dog caused by a European strain of *E. multilocularis* was also recently reported in British Columbia. **The Boxer dog in this case report had no reported travel history, suggesting that *E. multilocularis* is also present in Ontario**.

Foxes, and to a lesser extent coyotes, wolves and wild felids, are the principal definitive hosts wherein the adult tapeworm resides in the small intestine. Eggs passed in the feces of the definitive host are immediately infective for intermediate hosts. Within the intermediate host (mainly rodents such as voles, lemmings, and deer mice), the metacestode stage of *E. multilocularis* is an alveolar hydatid cyst that develops most often in the liver. Progressive budding and expansion of the cyst causes severe tissue damage and may also result in metastatic spread of metacestodes to other tissues. Domestic dogs are definitive hosts for the parasite (i.e., the adult tapeworm develops in their small intestine) but, in rare circumstances, may also develop the intermediate stage in their liver.

Humans are incidental hosts and may develop rare and potentially life-threatening alveolar hydatid cysts following inadvertent ingestion of infective eggs. These human infections are more commonly associated with shedding of eggs in dog feces rather than feces of wild canids resulting in infected domestic dog fecal-oral contact with humans (particularly children). To date, no zoonotic infections have been detected in association with this particular case. *AHL*

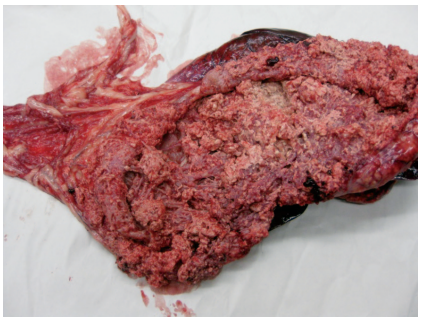

Figure 1. Alveolar hydatid cyst of *Echinococcus multilocularis* forming a large destructive lesion in the liver of a Boxer dog. (courtesy of Alicia Skelding)

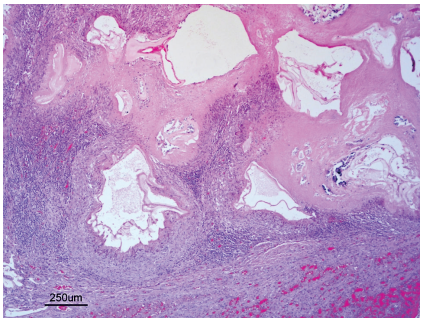

Figure 2. Multiloculated alveolar hydatid cyst causing extensive liver injury, and with extensive necrosis and chronic inflammation.