UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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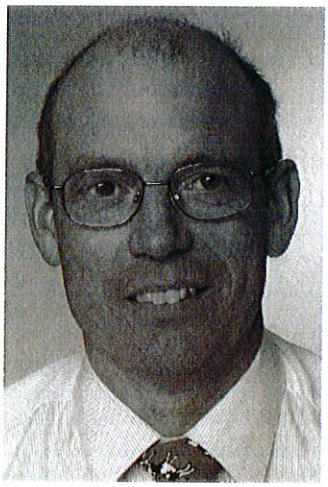
LAB REPORTS

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

Dr. Jim Fairles, AHL Client Services Veterinarian

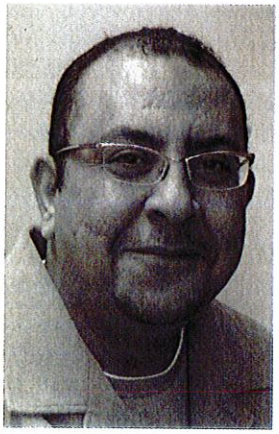


Dr. Fairles, as our new Client Services Veterinarian, will help client veterinarians to optimize their use of the services of the AHL, with emphasis on testing for domestic mammals, especially swine and cattle. This new position is an expansion of the Swine Health Advisor role filled by the recently retired Dr. Gaylan Josephson - Jim outlines his duties in his article on p. 35 of this newsletter.

Dr. Fairles holds degrees/certificates from the UofG - DVM, 1980; OVC Dairy Health Management Certificate Program, 1992; Master of Business Administration in Agriculture, 1999 - plus a Graduate Diploma in Business Management from Athabasca University, 1998. A partner for 22 years in Harriston-Mount Forest Veterinary Services, a 2-person mixed practice, Jim recently completed a 2-year assignment in China as Swine Veterinarian with CIDA on the China-Canada Animal Health Initiative.

Dr. Fairles can be reached by phone in Guelph at (519) 824-4120 ext 54611, by mobile phone at (519) 323-5558, by fax at (519) 821-8072, or by email at jfairles@lsd.uoguelph.ca We are delighted to have Jim join our team!

Congratulations Dr. Youssef!



We are pleased to announce that **Dr. Sameh Youssef, AHL Veterinary Pathologist**, passed the anatomic pathology examination of the American College of Veterinary Pathologists (ACVP) this fall, and is now officially a "**Diplomate ACVP**".

Dr. Youssef, who has earned DVM, MSc, and PhD degrees from the Alexandria Veterinary College, is currently completing his DVSc in anatomic pathology at the OVC. His primary interests are in diagnostic pathology of viral diseases and in surgical pathology. Dr. Youssef joined the AHL in September of 2002, and we congratulate him on his latest accomplishment.

Quick notes:

AHL Christmas hours, 2004 - Between Christmas and New Year's this year, the AHL will be open with limited staffing **9 AM - 5 PM, December 29, 30, and 31, (Weds, Thurs, Fri)**. Emergency necropsy service is available in Guelph on holidays except for Christmas Day.

(continued on next page)

Quick notes - *continued from cover*

- **Important update on SST tube interference for T4 and cortisol** - The manufacturer of our Immulite instrument has confirmed a positive bias of up to 20 -30% for total T4 and cortisol results, when samples are collected in BD Vacutainer SST glass and plastic tubes, compared to samples collected in BD Vacutainer glass (without gel) serum tubes. If you require further information, feel free to contact Susan Atkinson at (519) 824-4120 ext 54619, or satkinso@lsd.uoguelph.ca

- **New U of Guelph phone system** - The switchover is complete. If you encounter any problems with reaching us through the new system, please contact us at (519) 824-4120 ext 54538, or by email at holiver@lsd.uoguelph.ca

- **Recent proficiency testing results** - We have recently passed several external proficiency panels:
  - Clinical Pathology
    - VLA (Veterinary Laboratory Association), QA testing program (biochemistry, hematology, endocrinology, serology, and therapeutic drug monitoring parameters; results compared with up to 150 labs worldwide).
    - Bayer Urinalysis Proficiency testing study.
  - Immunology/Avian Virology
    - avian influenza virus agar gel immunodiffusion panel.
    - CWD/scrapie proficiency panel administered by CFIA/Nepean.
    - Johne's ELISA proficiency panel administered by the USDA.

- **Revised AHL submission forms are now available** - We have revised our forms to make them as user-friendly as possible. An order form is enclosed with this Newsletter. Please note on the order form if you would like us to personalize the forms for your clinic. The revised forms will also be posted on our website: http://ahl.uoguelph.ca

AHL Newsletter
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Editorial Assistant: **Ms. Helen Oliver**

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Its mission is to inform AHL clients and partners about AHL current activities, and lab-based animal disease events and disease trends.

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contributed to the generation of results reported in the AHL Newsletter.

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Jim Fairles, Client Services Veterinarian - my first month "on the job"

Jim Fairles *jfairles@lsd.uoguelph.ca*

I hope that everyone has enjoyed the very pleasant fall (and hopefully winter, as it will be winter when you read this!). I have the privilege of being the successful candidate for the "Client Services Veterinarian" position at the Animal Health Laboratory and have been in that position since October 1. Duties include front office and specimen reception management and a liaison between AHL client veterinarians and the AHL, with emphasis on swine and ruminant diagnostics. I come to AHL from 24 years of mixed animal practice background with emphasis on ruminant and swine health management, including recent experience as a veterinarian in Asia. The attraction of this position is partially based on my international experience where the veterinarian does not have the privilege of the backing of a recognized diagnostic laboratory. The complexities and disease syndromes of modern veterinary medical diagnosis make the availability of such a facility of paramount importance. Veterinary clients have come to expect increasingly more sophisticated diagnostic tests and procedures in both companion and food animal practice.

As a veterinarian from private practice, I believe (and certainly this was true in my case) that we take the services of a laboratory for granted. The practitioner expects timely and verified results, without putting much thought into the process of how this is accomplished. My first month here has provided much evidence that a concentrated effort is indeed made to ensure that the results that are conveyed to veterinarians are timely and accurate. Listed below are some of my experiences, which have removed some of those preconceptions that I have had about the lab.

Just as I was beginning my tenure here, I participated in a client meeting with the visiting assessors from the American Association of Veterinary Laboratory Diagnosticians. Just as OVC and veterinarians in practice have their clinics accredited, so do public laboratories. The AAVLD has currently accredited 43 public veterinary laboratories throughout North America. I also just recently returned from their annual conference, which provided a broad overview of the laboratory network in Canada and the United States. The AHL quality program is also ISO 9001:2000 registered, and when the next AAVLD accreditation is due, the lab will move to the ISO 17025 standard. **The bottom line is that the AHL is committed to QA /QC in order to provide accurate, timely and useful results.**

To get practical experience in the lab setting, I have been spending some time in Specimen Reception. Since we are dealing with biological systems, it is very difficult to have an SOP for every specific situation. Thus many questions arise about which testing procedure is required, cold chain concerns, improper labeling, improper transport media, improper samples .... all of which lead to slower turnaround times and the possibility of questionable results. So my one word of advice here is – **when in doubt, look in the May 2004 Users' Guide and Fee Schedule or CALL US**. We would be glad to answer any of your queries.

All of the AHL supervisors and staff are committed to "client service". As someone who has entered the laboratory environment with a "steep learning curve", I have been made to feel very welcome! This also mirrors my private practice experience with the AHL where everyone in their areas of expertise has been very approachable.

The lab has a wide variety of testing procedures, but due to limitations in economics and space cannot do everything "in-house". I was not aware of the extensive referral lab network that the lab employs. Currently, sending of animal biological samples to the USA can be onerous, and the lab expedites this process.

I look forward to serving you as a client advocate within the Animal Health Laboratory.

When dealing with complex systems and tasks, it is inevitable that a few things don't go quite right. This relates to the QA/QC comment above. Concerns are examined and acted upon to make sure that procedures are in place to minimize problems.

As the Client Services Veterinarian, my duties will be based on the following areas:

- Providing more detailed and specific answers about testing procedures, sample selection, limitations, results and further action in order for you, the veterinarian, to make the best use of the lab.
- To provide a contact liaison for the AHL for all of your problems, concerns and expectations.
- To help provide timely information in case coordination for you from all areas of the lab, and to work with you in detecting trends in animal disease.
- Provide information on the diagnosis and economics of disease and its relation to laboratory diagnosis so that you can better position your services as a health management professional. This is especially important in the economic environment of today's animal agriculture.
- Present timely information to you or your organizations on a wide variety of lab-related topics including sampling, testing procedures, and disease trends.

I look forward to serving you as a client advocate within the Animal Health Laboratory. Please feel free to contact me with any of your problems, questions or concerns. I am available to provide presentations to your organizations on any and all of the above topics. My experience here to date has been extremely positive, as this organization is truly client-centered. *AHL*

AHL Lab Reports

RUMINANTS

Malignant catarrhal fever in bison

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

All 27 bison imported from Alberta into a single herd in Ontario died within a few weeks of arrival; none of 20 resident bison in the herd died. Four dead bison were submitted to the AHL for necropsy.

Lesions included emaciation, meningoencephalitis, ulcerative keratitis, esophagitis, rumenitis, and ulcerative enteritis, with arteritis, hepatitis, splenitis and endometritis. A histologic diagnosis of malignant catarrhal fever (MCF) was made and confirmed by PCR in 2 of 2 bison tested.

American MCF of ruminants is caused by *Ovine herpesvirus 2* (OvHV-2) or *Caprine herpesvirus 2* (CHV-2). **Bison are very susceptible to MCF and death usually occurs within 8-48 hr of the onset of clinical signs** - separation from the herd may be the only clinical sign observed. Gross and microscopic lesions are less severe than those seen in cattle and lymphadenomegaly can be minimal.

Although outbreaks are usually associated with contact with sheep, the nearest sheep to this herd were approximately 2 km away; as was the case in another report in which 15 of 17 bison died of MCF. Bison to bison transmission has not been reported. *AHL*

References

O'Toole D, et al. Malignant catarrhal fever in a bison (*Bison bison*) feedlot, 1993-2000. J Vet Diagn Invest 2002;14:183-193.

Schultheiss PC, et al. Epizootic malignant catarrhal fever in three bison herds: differences from cattle and association with ovine herpesvirus-2. J Vet Diagn Invest 2000;12:497-502.

Highlights of camelid diagnoses from the AHL, 1998 to 2004

Janet L Shapiro, Phil Watson, Beverly Mc Ewen, Holly Lethbridge *jshapiro@kemptvillec.uoguelph.ca*

Between January 1, 1998 and August 31, 2004, 93 camelids, including 35 llamas and 58 alpacas, were submitted to the Guelph, Kemptville and Ridgetown locations of the AHL for necropsy (Table 1).

Abortions For 20 of the 21 camelid abortion submissions, no infectious etiology was found. However, 3/20 fetuses were in very thin to emaciated body condition, and 2/20 were diagnosed with fetal stress syndrome (*in utero* hypoxia). The dams of 2 fetuses for which no infectious cause of abortion was found had a history of systemic illness the day before aborting. A BVDV etiology was diagnosed for 1 alpaca abortion (details to follow in a subsequent newsletter article, by Carman *et al*).

1-day- to 14-day-old camelids Systemic bacterial infection, usually associated with omphalitis, was a common diagnosis in neonatal crias. Developmental anomalies diagnosed in this group included renal hypoplasia in an alpaca, and multiple congenital heart defects, consisting of ventricular septal defect and common pulmonic and aortic trunk, in a llama. Ventricular septal defects are reported as "relatively common" in llamas. Intestinal cryptosporidiosis, associated with a history of lethargy, colic and diarrhea, was diagnosed in multiple alpaca crias on 1 farm.

15-day- to 1-year-old camelids Lymphosarcoma was diagnosed in 1 llama and 2 alpacas aged 6-7 mo. The clinical course was reported as being only a few days duration. The llama was 1 of 4 in the herd that had been diagnosed with lymphosarcoma in the preceding 4 y. **Lymphosarcoma is reported as the most commonly diagnosed neoplasm of llamas and alpacas.** Other sporadic diagnoses were nutritional myopathy, heat stroke, systemic bacterial infection, peritonitis secondary to ileocecal intussusception, and perforated gastric ulcer. One 3-month-old llama with a history of being born with marked concavity of the distal neck and having an abnormal gait and stance, was found to have misshapen C5, C7 and T1 vertebrae, with dorsal displacement of T1, and compressive myelopathy of the spinal cord. A congenital malformation was suspected.

>1-year-old camelids **Digestive tract disease** was the most common diagnosis in this age group. Erosive stomatitis, negative for BVDV and other viruses, was diagnosed in 1 llama and 2 alpacas that were also diagnosed with mycotic gastritis and coccidiosis, respectively. Severe subacute

(continued on p. 37)

Camelid diagnoses *(continued from p. 36)*

erosive mucosal disease, negative for BVDV, was diagnosed in an alpaca that died while being treated for parasitic meningoencephalitis. Gastric ulceration was a frequent diagnosis, occasionally as the cause of death due to mycotic invasion or perforation with secondary septic peritonitis, but more commonly as an incidental finding in animals dying from other causes. Intestinal accidents were infrequently diagnosed and included idiopathic colonic impaction, and pyloric stenosis associated with pyloric fibrosis, ulceration and bacterial infection.

Neurological disease was the second most common diagnosis in camelids >1-y-old. Lesions consistent with parasitic meningoencephalitis due to "meningeal worm", *Parelaphostrongylus tenuis*, were found in 2 alpacas and 1 llama. Some affected herds reported multiple cases of parasitic meningoencephalitis in consecutive years. Polioencephalomalacia (3 alpaca cases), non-suppurative encephalitis and non-suppurative meningoencephalitis (the latter 2 diagnoses, in alpacas, presumed viral, but negative for rabies, West Nile virus, and/or malignant catarrhal fever) were also diagnosed. An alpaca with a 3-wk history of ataxia progressing to recumbency had spinal cord histopathology consistent with reported cases of degenerative myeloencephalopathy.

Hepatic disease was the third most common diagnosis. Hepatic lipidosis was the sole diagnosis in 1 adult alpaca, but was usually found in conjunction with other diseases such as septicemia, coccidiosis, intestinal impaction, and gastric ulceration. Parasitic hepatitis attributed to infection with *Fasciola hepatica* (liver fluke) was diagnosed in 1999 in an alpaca from southwestern Ontario.

Sporadic diagnoses in this group included exertional myopathy, idiopathic myocardial fibrosis, cardiac ventricular septal defect, bacterial myocarditis, and cardiac tamponade associated with shearing. Tumors were also diagnosed sporadically, and included granulosa cell tumor (alpaca), ruptured anaplastic (suspected adrenal cortical) carcinoma (alpaca), and stromal cell sarcoma causing mandibular osteolysis (llama). *AHL*

References

Cebra CK, et al. Lymphosarcoma in 10 new world camelids. J Vet Intern Med 1995; 9:381-5.

Fowler ME, ed. Congenital and hereditary conditions, *In* Medicine and Surgery of South American Camelids, 2nd ed., Iowa State University Press. 1998. 490.

Krogdahl DW, Thilsted JP, Olsen SK. Ataxia and hypermetria caused by *Parelaphostrongylus tenuis* infection in llamas. J Am Vet Med Assoc 1987;190:191-193.

Morin DE, et al. Degenerative myeloencephalopathy in two llamas. J Am Vet Med Assoc 1994;204:938-942.

Table 1. Camelid submissions by species and age group for each AHL lab

AHL location	Abortions		1 - 14-d-old		15-d - 1-y-old		>1-y-old		Total
	alpaca	llama	alpaca	llama	alpaca	llama	alpaca	llama	
Guelph	7	2	2	2	5	2	13	8	41
Kemptville	5	5	4	0	2	5	17	11	49
Ridgetown	2	0	1	0	0	0	0	0	3
Total	**21**		**9**		**14**		**49**		**93**

POULTRY

Inclusion body hepatitis update

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

Outbreaks of inclusion body hepatitis (IBH) in broilers, caused by fowl adenoviruses (FAdV's), have been occurring annually in southern Ontario. Various degrees of economic impact have been reported, but in some cases mortality in broilers has been as high as 25%. In the mid- and late 1990's, severe outbreaks of IBH also occurred in Alberta, and in the past two years similar problems have been regularly reported from all western provinces.

We reviewed cases with suspected FAdV/IBH involvement submitted to the AHL from January 1998 to October 2004 to determine the frequency of the disease. A "confirmed IBH case" was defined as a combination of a clinical diagnosis of IBH coupled with compatible histological lesions in the liver and/or virus isolation from the liver.

The clinical history typically consisted of a sudden rise in mortality with dopey, depressed birds with ruffled feathers. The age range of broiler chickens with IBH was 6 days to 7 weeks of age with most cases occurring at 3 to 4 weeks of age. At necropsy, livers were pale yellow to orange and mottled with red foci. Characteristic histopathologic changes included diffuse cytoplasmic vacuolation of hepatocytes, multifocal and coalescing hepatocyte necrosis, light inflammatory cell accumulations, and rare to numerous hepatocytes with an enlarged nucleus containing an amphophilic inclusion (Figure 1).

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IBH update (*continued from p. 37)*

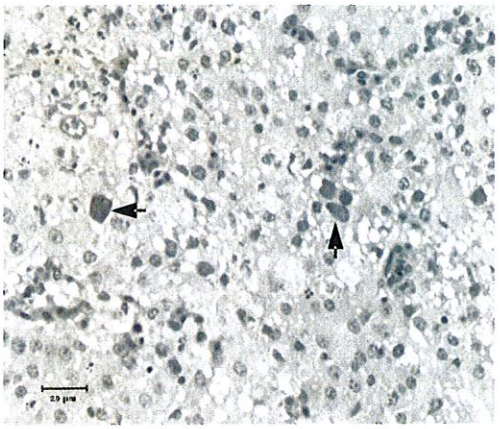

Figure 1. Hepatocellular vacuolation, focal necrosis, and large intranuclear inclusion bodies (arrows) in the liver of a bird with IBH.

We determined that, out of 245 submissions with FAdV involvement, the FAdV infection was IBH-related in 205 submissions (83.7%). The number of IBH cases varied from 16-65/year (Figure 2). **There has been a significant increase in the number of IBH cases during 2004.** The reason for the increase is partly because numerous cases from western Canada have been submitted to our laboratory. It is worth noting that, in 10.7% of IBH cases, other viruses have also been identified from other organs (6 infectious bronchitis virus (IBV), 14 reovirus, and 5 infectious bursal disease virus (IBDV)). However, the relevance of these agents to IBH is not known.

We found that 40 cases (16.3%) with FAdV involvement were not IBH-related and that other agents were the primary cause of disease. In most (16) non-IBH cases, FAdV's were isolated from the respiratory tract (lung/trachea). In 32.5% of non-IBH cases, there also was involvement of other viruses (11 IBV, 1 reovirus, 1 Newcastle disease virus, 4 IBDV). *AHL*

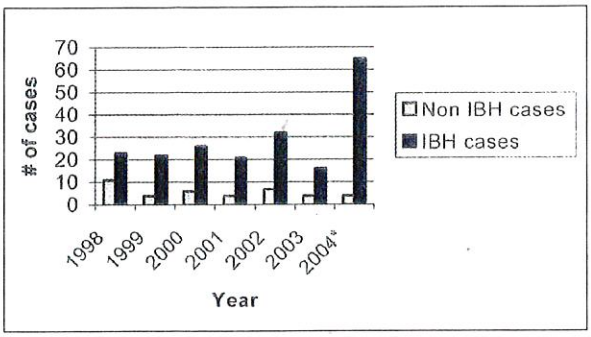

Figure 2. Number of submissions received by the AHL with FAdV involvement from 1998 to 2004 (*Jan - Oct) .

SWINE

Abortions associated with *Porcine circovirus* type 2

Tony van Dreumel, Beverly McEwen, John Hordyk, Josepha DeLay *tvandreu@lsd.uoguelph.ca*

Porcine circovirus type 2 (PCV-2) is mainly associated with **post-weaning multisystemic wasting syndrome** (PWMS). The condition was first reported from western Canada in 1995 and has since become worldwide in distribution. Other conditions that have more recently been associated with this virus include **porcine dermatitis and nephropathy syndrome** (PDNS), **proliferative necrotizing pneumonia** (PNP), and **fetal myocarditis.**

We report here a sudden outbreak of abortions associated with PCV-2 in a 100-sow non-vaccinated breeding herd. Gilts were home-raised; breeding was 90% by artificial insemination; occasional boars were bought (last one in June/04). Over a period of ~2 weeks, 13 sows aborted at 31-101 days gestation; 5 sows previously diagnosed as pregnant were subsequently found to be open, but abortion was not recognized in them. Aborted pigs from 3 litters at different stages of gestation (6-10 wks) were submitted for necropsy. There were no gross lesions in the fetuses. Microscopically, there was marked lymphoid depletion in lymph nodes from one of the fetuses. Numerous intracytoplasmic, amphophilic grape-like inclusions were evident in the affected lymph node (Figure 1). Immunohistochemistry (IHC) for PCV-2 antigen of the same lymph node was strongly positive (Figure 2). Pooled lung and kidney specimens from aborted fetuses were positive for PCV-2 using PCR. Other tests for abortifacient organisms - PCR for PRRS virus, FA for parvovirus, and bacteriology - were negative.

Since 1998, out of 363 submissions, we have identified 3 other cases of PCV-2 infection in aborted/mummified/stillborn pig fetuses. PCV-2 has been reported in stillborn and non-viable neonatal pigs with myocarditis, and was recently reported in 13.1% of 350 aborted/stillborn fetuses in South Korea.

We recommend that aborted/stillborn and non-viable neonatal pigs be tested routinely for PCV-2 by PCR using lung and thymus. In addition, lymphoid tissues (tonsil, thymus, lymph nodes, spleen) should be sampled for histopathology and IHC. *AHL*

Reference

Kim J, et al. Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. Vet Rec 2004;155:489-492.

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PCV-2 abortions *(continued from p. 38)*

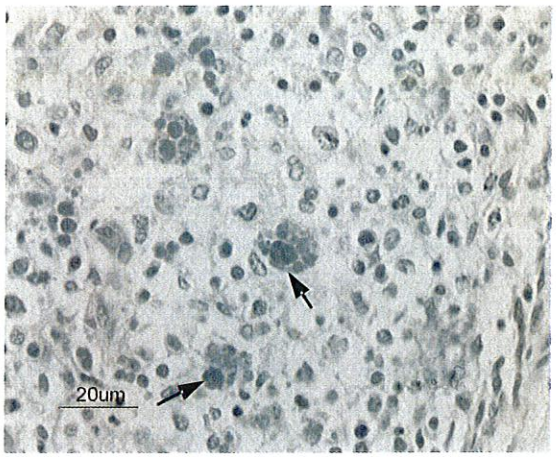

Figure 1. Lymphoid depletion and intracytoplasmic inclusion bodies (arrows) in fetal lymph node. H & E stain.

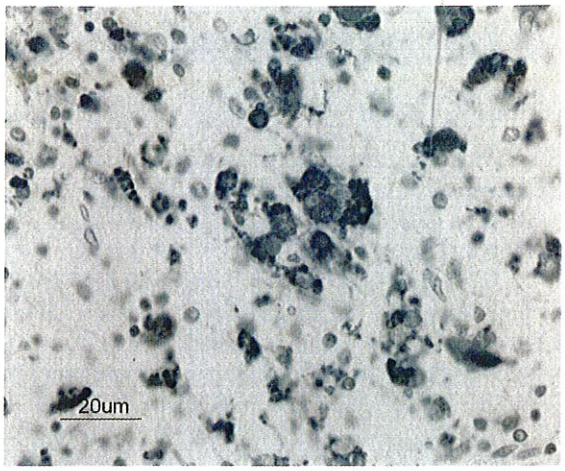

Figure 2. Abundant PCV-2 antigen in IHC-stained fetal lymph node.

Group A rotavirus infection in piglets in Ontario

Gaylan Josephson, Susy Carman, Jim Fairles *gjosephs@lsd.uoguelph.ca*

Group A rotaviruses are important enteric pathogens in many species, including pigs. A case control study in Ontario swine (1994-1997) showed that a relationship existed between management practices, including farm expansion with a higher proportion of gilts and low- parity sows, early weaning, and all-in/all-out production, and group A porcine rotaviruses. Herd size was larger and weaning age was younger (21 $\pm$ 4.3 days of age versus 24 $\pm$ 7 days of age) in rotavirus-positive herds. In addition, postweaning pigs raised in all-in/all-out nurseries were 3.4 times more likely to have a diagnosis of group A rotavirus.

A recent enquiry concerning the significance of the identification of a group A rotavirus in postweaning pigs led to a review of all group A rotavirus-positive AHL submissions received from January 1999 to the present. During this period, group A rotavirus was identified in 69 submissions, using the rotavirus latex agglutination test on intestinal scraping, colon content or fecal material. When non-autolyzed sections of small intestine were examined, villus atrophy was present in all cases, consistent with rotavirus infection. As described in the literature, atrophy was identified as being more severe in pigs under 2 weeks of age.

In this 1999-to-present review, the age of pigs on the date of submission ranged from 4 to 90 days, with an average age of 17.8 days. These figures are comparable to those found in the earlier 1994-1997 study, which had a range of 2 to 56 days of age, and an average age of 17.5 days. Age of piglets at time of onset of diarrhea was noted in 57 of these 1999-to-present submissions. **The number of affected cases was greatest for pre-weaned pigs within the first 15 days of life, as opposed to the 1994-1997 study, which found the highest number of affected cases in post-weaned pigs (>24 days of age).** The reasons for this change in affected age are not known, as management practices in these 1999-to-present rotavirus-infected herds were not studied. However, this may be related to continuing increase in herd size in Ontario, where levels of exposure and immunity are less uniform with larger numbers of gilts and low-parity sows. A previous AHL Newsle tter article has referred to chronic diarrhea in pre-weaned piglets, where group A rotavirus was identified.

In this 1999-to-present study, there were 25 group A rotavirus-positive submissions consisting of post-weaned pigs, with an average age of 28 days. In this group, there were 2 peaks of onset of diarrhea, one being the first 3-4 days after weaning and the second approximately 10 days post-weaning.

Of note, **other enteric pathogens were identified in 59 of the 69 submissions (85%)**, often with more than one additional pathogen per submission. These included 54 isolations of enterotoxigenic *E. coli* (ETEC), including strains of pool 1N, pool 2N, and K88 ETEC organisms. *Salmonella enterica* serovars Ohio, Agona, and Typhimurium DT104 were isolated on 10 occasions, coccidia were identified on 4 submissions, *Streptococcus suis* on 3 occasions, PRRS virus on 2 occasions, and *Clostridium difficile* toxin on 1 submission. The significance of these pathogens is unknown, but one can speculate that the severity of the illness increases with the number of identified pathogens. *AHL*

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Dewey C, et al. Relationship between group A porcine rotavirus and management practices in Ontario swine herds. Can Vet J 2003;44:649-653.

Reed R, Josephson G. The cost of not obtaining an accurate diagnosis. AHL Newsletter 2002;6:21.

Update on influenza virus isolates from Ontario swine, 1998-2004

Susy Carman, Christopher Olsen, Alexander Karasin, Beverly McEwen, Gaylan Josephson
scarman@lsd.uoguelph.ca

The AHL uses antigen detection ELISA, IHC, FAT, PCR and virus isolation in eggs and in cell culture to identify influenza virus in Ontario swine herds. Using all virology tests, we have detected 76 virus-positive cases in the past 7 years (Table 1).

It is important to type swine influenza virus, since pigs can act as "mixing vessels" for swine, avian and human strains of swine influenza virus. The AHL now types swine influenza virus isolates using multiplex PCR and has subsequently forwarded many isolates to the University of Wisconsin School of Veterinary Medicine for sequencing. Table 1 lists the number of cases for each type of influenza virus recovered from 1998 to Sept 2004 by the AHL and typed by either the AHL or the University of Wisconsin. In addition to H1N1 swine influenza viruses with all classical swine influenza genes, the AHL has recovered two H3N2 viruses similar to those found in Quebec swine, a wholly human H3N2 virus (recovered in 1997), a wholly human H1N2 virus, a wholly avian influenza H4N6, two wholly avian H3N3 viruses, and 2 reassortant H1N1 swine viruses with one human gene, from Ontario swine. Dual reassortant H1N2, triple reassortant H3N2 (Texas strain), and reassortant H1N1 viruses, similar to those found in swine in the USA, have not yet been found in Ontario swine. *AHL*

Table 1. Influenza virus-positive cases, and virus isolates by cell culture and egg inoculation, from Ontario swine, 1998 - Sept 2004

	Cases +ve by any test	Submissions/isolations			Numbers of each virus type isolated					
Year		Total	Positive	% positive	H1N1	H1N2	H3N2	H3N3	H4N6	Influenza A/untyped
1998	14	296	13	4%	12					1
1999	13	119	9	8%	7		1		1	
2000	8	91	6	7%	6					
2001	11	108	12	11%	9		1	2		
2002	10	77	12	16%	9					3
2003	11	94	8	9%	7	1				
2004 Jan-Sept	9	79	9	11%	8					1
Total	**76**	**864**	**69**	**8%**	**58**	**1**	**2**	**2**	**1**	**5**

AHL *Mycoplasma* PCR update

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Field validation of *M. hyopneumoniae* PCR

From 2002 to 2003, with the financial support of Ontario Pork and the OMAF Healthy Futures Program, we developed and evaluated a rapid PCR method for the detection of *M. hyopneumoniae* in swine lung tissue. Analytical validation and a small-scale field validation of the assay were completed. At the second stage of the project, with the support of Ontario Pork, the AHL performed a larger scale field validation of the PCR assay. This was completed in October 2004.

The field validation project compared the *M. hyopneumoniae* PCR to the *M. hyopneumoniae* fluorescent antibody test on 426 lung specimens from 220 post-mortem cases submitted to the AHL. The relative sensitivity, specificity, Youden's index, and kappa (agreement) between the tests were determined. Additionally, Bayesian methodology was used to determine the test accuracy in the absence of a gold standard.

At the specimen level, the diagnostic sensitivity of the *M. hyopneumoniae* PCR was 97.3% (probability interval 91.3, 99.9) and the diagnostic specificity of the *M. hyopneumoniae* PCR was 93.0% (probability interval 88.9, 97.1). At the case level, the diagnostic sensitivity of the *M. hyopneumoniae* PCR was 97.0% (probability interval 90.3, 99.9) and diagnostic specificity of the *M. hyopneumoniae* PCR was 92.2 (probability interval 85.8, 98.1).

We therefore conclude that the AHL *M. hyopneumoniae* PCR is a rapid, specific and sensitive assay for the detection of *M. hyopneumoniae* in lung tissue.

(continued on page 41)

AHL *Mycoplasma* PCR update *(continued from p 40)*

Field validation of milk *M. bovis* PCR

The AHL has developed a real-time PCR for detection of *Mycoplasma bovis* from milk samples (Figure 1). The assay has been analytically validated and is now being validated with field samples. The *M. bovis* real-time PCR is quantitative and can be completed in 3-5 hours, in contrast to culture isolation and identification that takes 3-7 days. **Once the validation is completed, the AHL will provide a new rapid test for milk *M. bovis*, an important contagious mastitis pathogen.** *AHL*

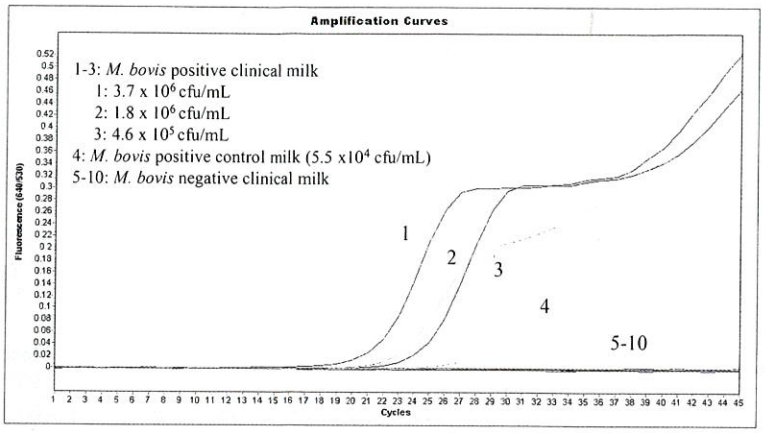

Figure 1. Example of *M. bovis* real-time PCR results.

HORSES

Pastern leukocytoclastic vasculitis in a 5-year-old Arabian mare

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A 5-year-old Arabian mare had chronic dermatitis characterized by ulceration and crusting of the distal white areas of 3 legs. The initial lesions affected the areas below the fetlocks, appearing clinically to be pastern dermatitis (scratches, mud fever), however, eventually involved two-thirds of the white areas of the affected legs. The affected areas were swollen and painful. Bacterial and fungal cultures, as well as skin scrapings for parasites, were all negative. Treatment with topical and systemic antibiotics, antifungal, anti-inflammatory and anti-parasitic preparations resulted in little or no improvement.

Several sections of skin were submitted for histopathology. The lesions were similar in all sections. There was marked necrosis, erosion, and ulceration of the epidermis. In addition, there was marked parakeratosis, acanthosis and edema with vesicle formation of the epidermis. Necrosis of the walls of some dermal vessels with a mild perivascular inflammatory cell reaction was also evident. The inflammatory cells consisted of a mixture of neutrophils and monocytes. There was also marked hypertrophy of the endothelial cells of the affected vessels (Figure 1).

Pastern leukocytoclastic vasculitis has been described as a relative common but poorly understood specific entity unique to the horse that affects only unpigmented distal extremities. The disease affects adult horses during the summer months and appears to be photoactivated.

Recommendations for the clinical management of the disease are provided in the reference. This mare was immediately treated with high doses of prednisolone and maintained on gradually decreasing doses for a month. Treatment was discontinued for the following 3 weeks. The mare is currently maintained on low dosages of prednisolone. The lesions have almost completely healed. *AHL*

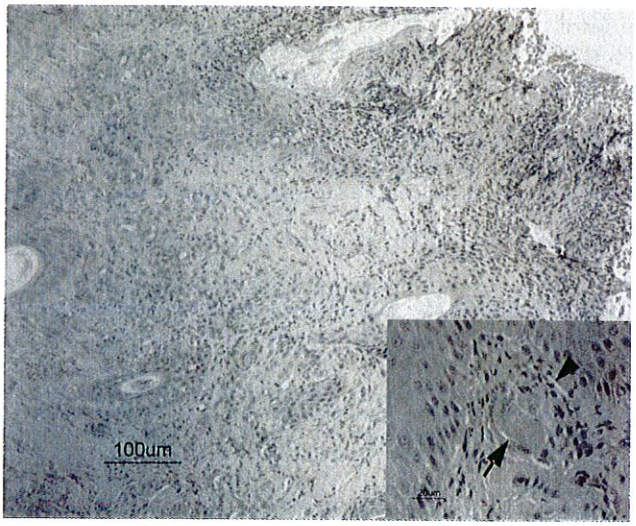

Figure 1. Necrosis, erosion and ulceration of epidermis, and perivascular inflammation. Inset: thrombosis (arrow) of a dermal vessel with leuocytoclasis (nuclear dust) in its wall (arrowhead).

Reference

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Equine neurologic disease surveillance 2004

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A surveillance project to identify Ontario horses with eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) infection began on August 1, 2004 and was funded jointly by the Ontario Ministry of Agriculture and Food and the Ontario Ministry of Health and Long-Term Care, with the cooperation of the Canadian Food Inspection Agency. The goal of this project, which is ongoing, is to identify geographic areas in which horses are infected with these viruses in an attempt to anticipate and prevent human infections. Horses are effective sentinels of both diseases, often becoming infected prior to humans living in the same environment.

As of October 21, 68 equine cases had been submitted to the Animal Health Laboratory under this program. These cases included 49 live horses for which serology for WNV and EEEV was performed, and 19 dead horses from which either carcasses or tissues were submitted for necropsy, histopathology, and EEEV and WNV PCR testing on brain. Of the dead animals, 16 were tested for rabies by CFIA and were negative. Six horses were serologically identified as acutely infected with WNV, based on a positive WNV IgM ELISA and compatible clinical signs, and **1 horse was acutely infected with EEEV** based on a similar test and associated history. Four additional horses were serologically identified as WNV-positive at another laboratory, for a **total of 10 Ontario horses with acute WNV infection in 2004**. Of these 10 horses, 7 were not vaccinated against WNV, 1 had an unknown vaccination history, 1 had received an initial vaccination but no booster vaccination, and 1 had been vaccinated less than 2 weeks prior to the onset of clinical signs. The single EEEV-infected horse was unvaccinated for EEEV.

Submission of surveillance samples continues but has decreased as mosquito season tapers off. A final summary of results of this surveillance project will be available in the March 2005 AHL Newsletter.

It is very important that practitioners include all required information on cases such as these, as WN fever and EEE are now classified as 'immediately notifiable diseases'. Under the federal Health of Animals Regulations, all laboratories making a diagnosis of a disease set out in Schedule VII must report immediately to the CFIA. The laboratory must include:

a) the **name, address and telephone number** of the person who owns or has the possession, care or control of the animal,
b) the location of the animal (**including township and postal code**), and
c) all other information that the laboratory has in relation to the animal.

This information is essential, as this database on immediately notifiable diseases will be used to certify products or animals based on geography. **The AHL is required to forward this information to the CFIA when diagnostic tests for WNV and EEE are positive.** *AHL*

COMPANION ANIMALS

Toxic shock syndrome and necrotizing fasciitis associated with *Streptococcus canis* infection in 2 dogs

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Case 1: A 3.5-year-old mixed-breed dog was presented with a history of acute death with no previous known illness. Necropsy findings included acute thymic hemorrhage, hemothorax and ~50 mL of serofibrinous turbid pericardial fluid. The differential diagnoses included peracute bacterial septicemia and rodenticide toxicosis. Histopathologic findings included pulmonary hemorrhage, acute renal tubular necrosis, and patchy multisystemic petechiae. Rodenticide toxicology on liver and stomach contents was negative, and a pure culture of *Streptococcus canis* confirmed the diagnosis of *S. canis* septicemia.

Case 2: A 5-year-old male Labrador retriever with a 1-wk history of treatment with prednisone and topical antibiotics for facial pyodermatitis died within 24 hr of becoming acutely ill with cellulitis of one, and then both, forelegs. Treatment with orbifloxacin, enrofloxacin and prednisone was ineffective. On gross necropsy, there was severe bilateral foreleg cellulitis, and on histopathology there was severe acute cellulitis with many coccoid bacteria in affected areas. *S. canis* was isolated from subcutaneous tissues and muscles of both legs, but not from the spleen.

During the last 2 decades, highly virulent strains of *S. canis* have emerged as an important cause of "toxic shock syndrome" (TSS) and necrotizing fasciitis (NF). In 1996, TSS was reported in several dogs from southern Ontario associated with *S. canis* infection; in some cases infection was associated with NF, and the isolates produced protease and a CAMP-like factor. *(continued on p 43)*

S. canis toxic shock *(continued from p 42)*

Fluoroquinolone antibiotics (ciprofloxacin, enrofloxacin, orbifloxacin, etc.) can cause modulation of *S. canis* virulence factors and production of superantigen leading to TSS and NF. A recent study has shown that enrofloxacin can induce an endogenous bacteriophage (virus) to lyse *S. canis*, and that this bacterial virus encodes a novel mitogenic protein related to pokeweed mitogen. Release of this virus-encoded mitogen may be the reason that about half the cases of severe *S. canis* in dogs are associated with fluoroquinolone use. *AHL*

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Miller CW, et al. Streptococcal toxic shock syndrome in dogs. J Am Vet Med Assoc 1996;209:1421-1426.

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Comparison of two blood-counting systems for analysis of blood of common domestic animals

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Many reference veterinary laboratories in North America use a large hematology system such as the Bayer Advia 120 as their primary hematology system. With many smaller clinic hematology systems available, we were interested in determining how the performance of a newer small hematology analyzer, the VetScan HMII (Abaxis), would compare to the larger system for the analysis of canine, feline, and equine blood samples. The HMII uses impedance methodology, and the Advia utilizes laser-assisted flow cytometry with two different methods (measuring absorption and scatter), namely a peroxidase staining method, as well as a basophil channel that measures nuclear density against cell size. These two instruments may often be used in conjunction with one another for hematology analysis by veterinarians who routinely use a smaller clinic system in-house as well as the reference laboratory for more complicated cases and as quality assurance for the in-clinic lab. The objective of this study was to compare the results of the standard hemogram on the clinic system with the reference instrument.

Samples that had previously been run on the Advia 120 (134 canine, 47 feline, 91 equine) were run twice on the VetScan HMII in the Clinical Pathology Laboratory at the AHL. Samples were submitted in EDTA blood vials, received from the Ontario Veterinary College's Veterinary Teaching Hospital and external clinics, and analyzed within 2 hours of each other.

The parameters compared in this study were concentrations of white blood cells (WBC), granulocytes (neutrophils, eosinophils, basophils), red blood cells (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), and platelets (Plts). The HMII and the Advia hematology analyzers showed excellent agreement for the 7 parameters that were compared. Correlation between both systems met with almost perfect agreement. The exception was equine platelet counts that showed moderate agreement. The feline platelet counts showed substantial agreement. This reduced correlation can be attributed to platelet clumping as confirmed on the blood smear. Correlation coefficients were calculated to compare paired sample values in each species and reported in Table 1.

The HMII and the Advia employ notably different methodologies. This may have some effect on the measured level of agreement of the various parameters. The carryover, within-run precision, run-to-run precision, and linearity of the HMII was acceptable for all species and parameters. **With the exception of the platelet parameter in horses, the HMII was a reliable analyzer when compared with the Advia and would be an asset as an in-house system in a veterinary practice.** *AHL*

Table 1. Concordance correlations for canine, feline and equine blood samples tested with the VetScan HMII and the Advia 120

Analyte	Concordance correlation		
	Canine	Feline	Equine
WBC	0.99	0.88	0.98
Granulocytes	0.95	0.92	0.92
RBC	0.95	0.96	0.89
Hgb	0.97	0.97	0.96
Hct	0.96	0.92	0.88
MCV	0.80	0.85	0.93
Plts	0.81	0.77	0.41

Concordance correlation is interpreted as a kappa value: <0.20 = slight, 0.20 – 0.40 = fair, 0.40 – 0.60 = moderate, 0.60 – 0.80 = substantial, >0.80 = almost perfect.

Mushroom toxicosis in a young dog

Josepha DeLay, Brent Hoff, Margaret Stalker, Susan Fink *jdelay@lsd.uoguelph.ca*

A 6-month-old male Golden Retriever pup was presented to his regular veterinarian due to vomiting and diarrhea of a few hours duration. Within the 3 days preceding presentation, the pup had had several episodes of eating foreign material, including cherry tomatoes that resulted in a period of gagging and dyspnea (presumably from esophageal obstruction) and a child's sandal, resulting in a previous bout of diarrhea. The major differential diagnosis at the time of final presentation was ingestion of yet another foreign body; however, the dog's condition deteriorated very rapidly in the few hours following admission to the veterinary hospital, culminating in a cluster of short seizures, coma, and death.

At necropsy, the wall of the gall bladder was severely and uniformly thickened, and multifocal hemorrhage was evident in myocardium and urinary bladder. Histologically, the most significant lesions were present in liver, with massive, acute coagulation necrosis of hepatocytes but retention of portal structures and minimal inflammation. **Acute hepatotoxicity was the primary differential diagnosis for liver damage of this magnitude, and both blue-green algae and *Amanita* mushrooms were high on the list of potential intoxicants.**

Histologic examination of vomitus from this pup identified distorted gill structures with a few fungal hyphae, similar to the histologic appearance of a control mushroom but assuming that some loss of tissue detail had occurred due to partial digestion and exposure to gastric acid. A thorough examination of the owner's property identified mushrooms consistent with the distinctive appearance of *Amanita muscaria* in an area to which the pup had access (Figure 1; see color photo on the Web at http://ahl.uoguelph.ca); several other, smaller and relatively non-descript mushrooms were also found, which may have represented other *Amanita* spp.

The toxic principles of *Amanita muscaria* include ibotenic acid and muscimol, which affect both the autonomic and central nervous systems and may have resulted in some of the clinical signs identified in this dog. Mushrooms of the more toxic *Amanita phalloides* group contain several toxins, of which the cyclopeptide amanitins are the most potent. Lesions identified histologically suggest that these species were more likely involved in the demise of this dog. These toxins inhibit RNA polymerase in cells, preventing protein synthesis and resulting in cell death. Tissues with the highest mitotic rate, such as liver and intestinal crypt epithelium, are most severely affected, with renal involvement if the animal survives the initial liver insult.

Figure 1. *Amanita muscaria* mushroom.

In humans, a single mushroom cap can contain sufficient toxin to be lethal for an average-sized adult. Severe liver damage in this dog's case was typical of that produced by amanitins. **We see approximately 2 to 3 canine cases of severe hepatic damage due to mushroom toxicosis annually at the AHL.**

Most dogs develop clinical signs within 6-24 hours of ingestion of *Amanita phalloides*, with vomiting, diarrhea (which is sometimes bloody), and abdominal pain. Some animals appear to recover for several hours but then deteriorate rapidly over 2-3 days, as in this dog's case, due to fulminant hepatic necrosis. Coagulopathy and encephalopathy are common, as seen here, and are due to hepatic failure.

Treatment is often unsuccessful unless instituted early in the course of disease, and consists of evacuating the dog's stomach, administration of activated charcoal to prevent further absorption of toxins, and administration of penicillin G or silibinin in an attempt to decrease hepatocellular uptake of amanitins. Muscarinic effects following ingestion of *Amanita muscaria* are poorly documented in animals but can include drooling, abdominal pain, diarrhea, bradycardia, and pupillary constriction; other CNS signs such as ataxia and coma can occur due to concurrent effects of ibotenic acid. *AHL*

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Tegzes JH, Puschner B. Toxic mushrooms. Vet Clin North Am Small Anim Pract 2002;32:397-407.

Newsletter Figures are posted in full color in the Web version of the Newsletter - http://ahl.uoguelph.ca