UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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Visit our website at http://ahl.uoguelph.ca

For access to AHL lab results via the Web, please contact Robin Simkin, (519) 824-4120, ext 54530 to activate your password.

Dr. Kris Ruotsalo, new AHL Veterinary Clinical Pathologist

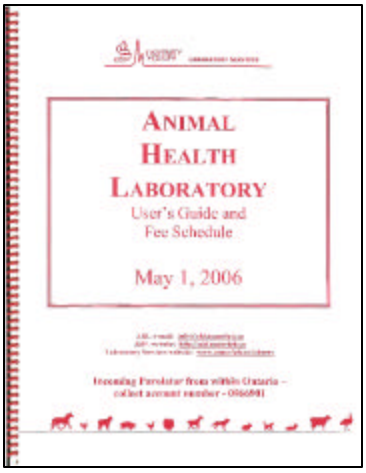
Dr. Kristiina Ruotsalo joined the AHL on April 3, 2006, in the new position of Veterinary Clinical Pathologist in the AHL. Kris completed her DVM at OVC in 1990, her DVSc in 1996, and became a Diplomate of the ACVP in Clinical Pathology in 2004. She brings with her experience in private mixed and small animal practice as well as in private veterinary labs.

Please join me in welcoming Kris to Lab Services and the AHL!

Dr. Ruotsalo joins **Dr. Brent Hoff**, whose responsibilities include clinical pathology and clinical toxicology on a 50:50 basis.

May 1, 2006, AHL User's Guide and Fee Schedule

We hope that this comprehensive source of information will save you time and help you make the most of our services. The Guide/Schedule is also available on the Web at http://www.labservices.uoguelph.ca/units/ahl/files/AHL-userguide.pdf http://www.labservices.uoguelph.ca/units/ahl/files/AHL-tests-and-fees.pdf



We have adjusted some of our fees to reflect increasing costs of materials. We have added some new tests to the test menu and have dropped some tests that have been used infrequently or that have become outmoded. This is part of our ongoing effort to make the best use of testing resources to bring you services that are current and meet today's needs.

We added **tabs** this year to better identify the **Indexes** of tests by species, as well as tests by discipline, and sub-divided our tests into **Farm animal tests** and **Companion animal tests.**

For clients in specialty practice, we would be happy to provide the Fee Schedule for you sorted by test name, method, etc., within your species of primary interest. Please contact Helen Oliver at (519) 824-4120, ext 54538.

We will continue to strive to provide the most cost-effective testing strategies to help you deal with animal health challenges, and will keep you posted on changes in test availability and fees through this quarterly AHL Newsletter - also available on the Web at http://www.labservices.uoguelph.ca/units/ahl/news_notes.cfm#News *AHL*

Clinical Pathology biochemistry mini-profiles

Kris Ruotsalo, Brent Hoff

Profile	Code	Fee ($)	Tests
Small animals			
Geriatric 1	gprf1	44.00	Full CBC + T4 + Ca, Na, K, Cl, anion gap, TP, alb, glob, A:G ratio, urea, creatinine, glucose, ALP, S-ALP, ALT, Na:K ratio
Geriatric 2	gprf2	32.00	T4 + Ca, Na, K, Cl, anion gap, TP, alb, glob, A:G ratio, urea, creatinine, glucose, ALP, S-ALP, ALT, Na:K ratio
Hepatic	hpsmp	20.25	TP, alb, glob, A:G ratio, urea, glucose, cholesterol, T bilirubin, ALP, S-ALP, ALT, GGT, bile acid
Renal	rnsmp	20.25	Ca, P, Na, K, Cl, CO_2, anion gap, TP, alb, glob, A:G ratio, urea, creatinine, glucose, Na:K ratio, calc osmo
Presurgical	pssmp	19.00	Na, K, Cl, TP, urea, creatinine, glucose, ALP, GGT, ALT, Na:K ratio
Presurgical 1	psmp1	22.00	Advia CBC + TP, urea, creatinine, glucose, ALP, GGT, ALT
Presurgical 2	psmp2	18.00	TP, urea, creatinine, glucose, ALP, GGT, ALT
Horses			
Renal	rnlmp	20.00	Ca, P, Mg, Na, K, Cl, CO_2, anion gap, TP, alb, glob, A:G ratio, urea, creatinine, glucose, Na:K ratio, calc osmo
Hepatic	hplmp	20.00	TP, alb, glob, A:G ratio, urea, glucose, cholesterol, T bilirubin, GGT, AST, CK, GLDH, bile acid
Presurgical	pslmp	20.00	Na, K, Cl, TP, urea, creatinine, glucose, GGT, AST, CK, Na:K ratio

Note: CBC (counts and differential) can be added to any of these mini-profiles for an extra charge of $10.00.

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Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistant: **Ms. Helen Oliver**

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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.*

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Results of AHL submission-form animal demographics questionnaire

Bruce McNab, Jim Fairles

A 1-page questionnaire was included with each copy of the AHL Newsletter mailed to approximately 875 veterinarians across Ontario, in late February 2006. **The questionnaire asked farm-animal practitioners for input on the collection and use of animal demographic information on AHL submission forms.**

A total of 57 completed questionnaires were returned to OMAFRA. The geographic distribution of responses included 12, 15, 27 and 3 returns from veterinarians indicting the first letter of their postal code as K, L, N or P respectively, across Ontario. By type-of-practice, 8 questionnaires were returned from veterinarians involved primarily in bovine practice, none was received from primarily swine, 4 from poultry, 7 from equine, 4 from various farm animal practice or other, and 34 from mixed farm/companion animal practitioners.

Farm-animal practitioners were asked to indicate their level of disagreement or agreement with each of the following six statements by circling one number on a linear scale from 1 to 5, where 1 represented strong disagreement, 3 represented mild agreement, and 5 represented strong agreement with the respective statement. The 6 statements were: i) I believe summary and trend statistics of farm-animal submissions to AHL have value to industry and government; ii) I would like to see more AHL summary statistics and trend-charts in AHL or OMAFRA publications; iii) Such statistics are useful to me and my clients because they help put my practice and client findings into context; iv) I believe completing the demographics section of forms is a reasonable requirement for farm-animal submissions, in part because government tax dollars are used to keep AHL food-animal test prices down; v) I find parts of the demographic section of AHL forms to be unclear or difficult to complete. (if so, please explain why in comments section below); and vi) Regardless of form-clarity or the utility of the data, I do not wish to provide demographic information for reasons of client confidentiality.

For each of the above statements, Table 1 summarizes the number (frequency) of replies for each agreement score, the average score by practice type, and the overall average agreement score.

These results suggest that, in general, respondents saw: a) utility in animal demographic data for government, industry and practitioners, b) thought that collection of such data was a reasonable request, c) found AHL submissions forms to be reasonably clear but not as clear as they might be, and d) were not averse to providing demographic information.

Written comments are paraphrased as follows: a) it can be difficult to obtain animal demographic information; b) it can be difficult [time-consuming] to obtain postal code of farm; c) it's more important to capture municipality or county; d) overwhelmed by variety of tests available for each disease; e) difficult to choose most appropriate test; f) demographic data sometimes seems redundant but can still see need; g) need more space to write on submissions forms; h) equine form too oriented towards bovine; i) Newsletter is of value to regulatory work; j) should not switch from positive to negative statements part way through questionnaire; k) should include stamped self-addressed envelope with survey to encourage returns; l) variability in sample submission rates between practices will skew interpretation of submission demographics. *AHL*

Table 1. Summary of AHL submission-form animal demographics questionnaire

Statement summary	Frequency by agreement score 1 = strongly disagree 5 = strongly agree					Average agreement score by practice type					Overall average agreement score
	1	2	3	4	5	Bovine n=8	Poultry n=4	Equine n=7	Farm animal n=4	Mixed practice n=34	
i. has value to industry and government	0	1	4	24	28	4.8	4.5	4.1	4.0	4.4	**4.4**
ii. want to see more in publications	2	1	9	25	20	4.3	4.5	4.0	3.5	4.0	**4.0**
iii. useful to practitioners	1	3	9	23	19	4.5	4.5	3.7	3.0	4.0	**4.0**
iv. providing demographics is useful	0	0	14	23	19	4.5	4.3	3.7	4.0	4.0	**4.1**
v. parts of AHL form are unclear	15	27	6	4	1	1.5	2.0	2.4	3.3	2.1	**2.1**
vi. do not wish to provide demographics	27	25	2	0	1	1.4	2.3	2.0	1.6	1.5	**1.6**

AHL Lab Reports

RUMINANTS

Ovine abortion diagnoses, 1998 - 2006

Beverly McEwen, Josepha DeLay

Causes of ovine abortions have not changed substantially over 8 years (Table 1). Of these, 54 (19%) were *Toxoplasma sp*., 48 (17%) were *Chlamydophila abortus* (formerly *Chlamydia psittaci*), 13 (5%) were *Coxiella burnetii*, and 10 (4%) were *Campylobacter spp*. abortions.

Several of these agents can cause **serious infections in people** and care must be taken when handling and packing sheep and goat abortion specimens. Fetuses or fresh /frozen tissue samples should be double-bagged in sealed plastic bags to prevent fluid leakage from contaminating packaging and infecting clinic, courier, and lab staff handling the packages. **Pregnant women should not handle tissues from sheep or goat abortions.**

The chance of obtaining an etiologic diagnosis greatly increases with appropriate sampling. Because **diagnostic lesions are more frequent in small ruminant placentas than in fetuses,** it is very important to submit placenta as well as brain, parenchymal organs, skeletal muscle and intestines for histopathology and selected tissues for microbiology (see the AHL User's Guide for details). *AHL*

Table 1. Pathology diagnoses, ovine abortion cases, March 1998 – March 2006.

	98/99	99/00	00/01	01/02	02/03	03/04	04/05	05/06	Total (%)
Idiopathic	21	37	16	15	8	12	16	10	135 (48%)
Toxoplasma sp	12	4	13	9	2	4	3	7	54 (19%)
Chlamydophila abortus	8	9	7	4	6	3	3	8	48 (17%)
Placentitis	1	6	4	5	3	5	0	0	24 (9%)
Bacteria (miscellaneous)	0	4	5	3	3	1	0	3	19 (6%)
Stillbirth	3	0	8	2	3	1	0	0	17 (6%)
Campylobacter spp.	0	2	2	1	3	2	0	4	14 (4%)
Coxiella burnetii (Q fever)	1	1	1	3	1	0	2	4	13 (5%)
Infectious, etiology unknown	5	2	1	0	0	0	0	0	8 (3%)
Other (mummified fetus/anomalies)	4	0	0	0	0	3	0	0	7 (2%)
Bacillus licheniformis	1	0	0	0	0	0	0	0	1 (0.3%)
Salmonella Arizonae	0	1	0	0	0	0	0	0	1 (0.3%)
Salmonella Tennessee	1	0	0	0	0	0	0	0	1 (0.3%)
Total	**57**	**66**	**57**	**42**	**29**	**31**	**24**	**36**	**342**

Congenital goiter in dairy calves

Tony van Dreumel, Andrew MacLeod

Over a 2-week period, 22 calves were weak at birth and 10 died within 1-2 hours after birth; 12 calves survived. Affected calves had markedly swollen necks especially in the thyroid area. The weak calves were lethargic, had forceful respiration, were unable to nurse and had to be tube fed. All affected calves were from first-calf heifers kept on a separate farm from the milking cows.

Four live day-old calves were submitted to the AHL for necropsy. The calves were semi-comatose, had large swellings of the proximal ventral neck extending to the thoracic inlet, and marked subcutaneous edema of the ventral neck. The thyroids were greatly enlarged (Fig. 1), and weighed from 92-204 g (normal range=8-12 g). Microscopically, the thyroid follicles were hyperplastic and few contained colloid. The history, gross and microscopic findings were compatible with a diagnosis of **congenital hyperplastic goiter**. Additional history indicated that the heifers were fed hay and corn that was not supplemented with iodized salt.

The Great Lakes region is notoriously deficient in iodine, and this case again illustrates the importance of adequate supplementation of iodized salt to pregnant animals to prevent goiter in their offspring. *AHL*

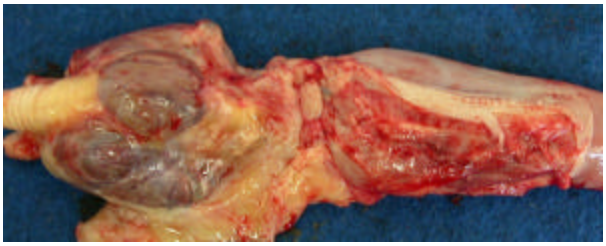

Fig 1. Greatly enlarged thyroid in a calf with congenital goiter.

POULTRY

Characterization of field isolates of infectious laryngotracheitis virus from Ontario

Davor Ojkic, Janet Swinton, Marie Vallieres, Emily Martin, Jan Shapiro, Babak Sanei, Brian Binnington

A sudden increase in the number of cases of infectious laryngotracheitis virus (ILTV) infection occurred in the fall of 2004 when 5 broiler flocks in Niagara Peninsula were diagnosed with ILT. At about the same time, 2 more ILT cases occurred in eastern Ontario and one in a backyard layer flock in the Waterloo Region (Figs. 1 & 2). ILT cases from eastern Ontario were believed to be a result of adverse vaccine reactions, but there was speculation that the Waterloo Region ILT case was linked to the Niagara Peninsula ILT outbreak, and that the outbreak was spreading.

Objectives of this study were to: (i) examine the relatedness among ILT viruses from Niagara Peninsula at a molecular level; (ii) determine whether ILT viruses from Niagara Peninsula were related to other Ontario field isolates or not; (iii) determine whether ILT viruses from Niagara Peninsula were related to 4 vaccine viruses or not. Results of molecular analysis would also provide objective data to confirm or disprove the hypothesis that the case from Waterloo Region was somehow linked to the Niagara Peninsula outbreak.

In summary, we used PCR-RFLP analyses of ICP4 and glycoprotein E genes and partial sequencing of UL47/glycoprotein G genes to examine at a molecular level ten ILTV field isolates from 2004 and early 2005 and four ILT vaccine viruses. We determined that:

1) Niagara Peninsula ILT viruses were identical amongst themselves, because there were no detectable differences among these isolates at a molecular level.

2) Niagara Peninsula isolates were at a molecular level different from other field isolates that were examined during this study.

3) Niagara Peninsula isolates were at a molecular level different from all 4 vaccine viruses that were examined.

Our results also disproved the hypothesis that the Waterloo Region layer flock was somehow a part of, or linked to, the Niagara Peninsula ILT outbreak. We demonstrated that **the Niagara Peninsula outbreak was an independent cluster of ILT not related to other cases that occurred in Ontario during 2004 and early 2005**. The origin of the ILTV that caused the Niagara Peninsula outbreak remains unknown.

(A full-length article has been accepted for publication in *Avian Pathology*.) *AHL*

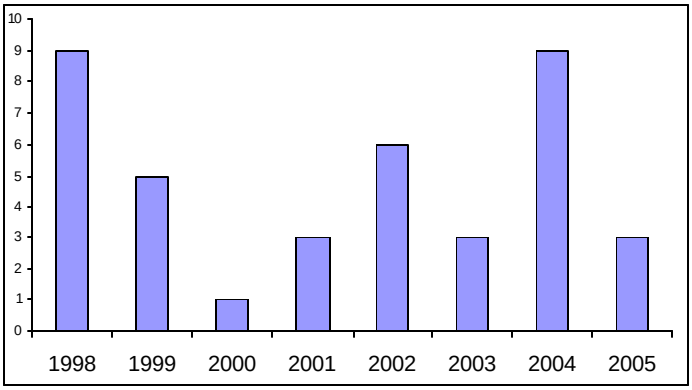

Fig. 1. Infectious laryngotracheitis cases (histopathology and/or virus isolation) in poultry in Ontario, 1998-2005.

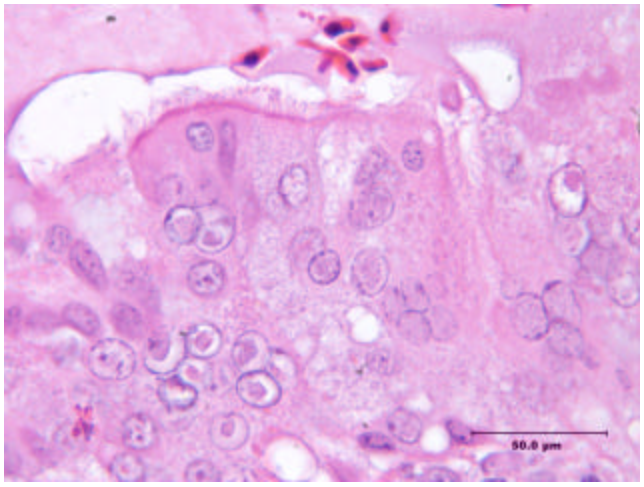

Fig. 2. Tracheal epithelial cells with intranuclear herpesviral (ILTV) inclusion bodies.

SWINE

Porcine circovirus type 2 - associated disease diagnoses continue into 2006

Susy Carman, Beverly McEwen, Josepha DeLay, Hugh Cai, Jim Fairles

***Porcine circovirus* type 2 (PCV2)-associated disease continued into 2006**, with 154 new cases presented from Jan-Apr 2006. The percent total of swine submissions increased from 8.9% in 2005 to 10.9% for the first part of 2006 (Fig. 1). Because these data are impacted by submission biases to the diagnostic laboratory, they cannot be regarded as population prevalence estimates.

The PCV2 PCR-RFLP typing for all PCR testing requests continued to show a significant change from RFLP type 422 seen in previous years to RFLP type 321 (Fig. 2), although RFLP type 422 is still present within Ontario swine. This consistent change in RFLP typing patterns reflects a consistent change in the gene sequence recognized by two restriction enzymes. Ontario RFLP type 321 viruses continue to have greater than 99% sequence homology with each other, and to have 98% sequence homology with those reported for the UK, France and China. These Ontario viruses have only 91.6% sequence homology to the previously dominant Ontario RFLP type 422 viruses ,and are only 92-93% similar to those previously reported from the USA. *AHL*

References

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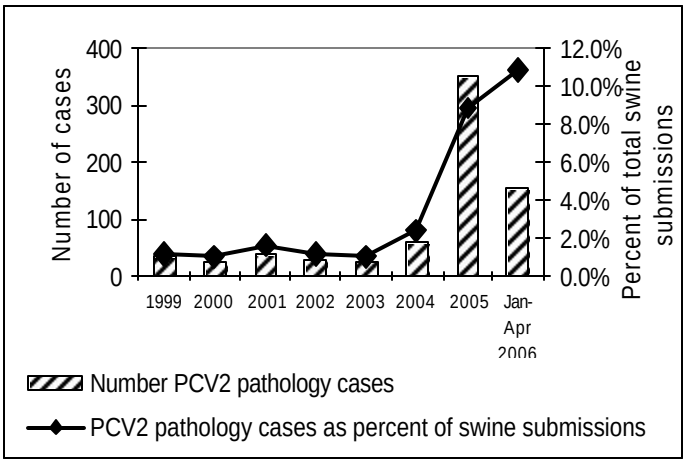

Figure 1. Number of PCV2 pathology cases, and PCV2 pathology cases as percent of total swine submissions, 1999 - 2006.

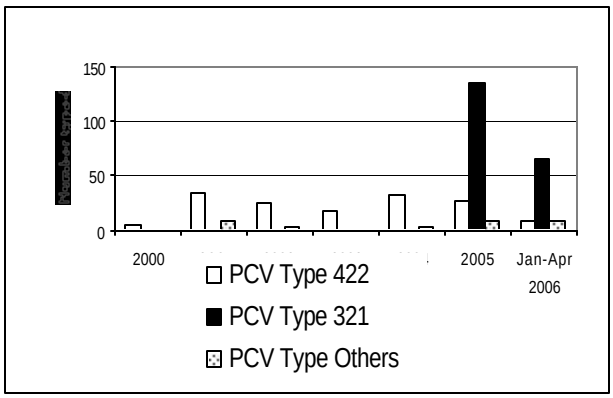

Figure 2. PCR-RFLP typing of PCV2, 2000 - 2006.

Update on influenza virus isolates from Ontario swine, 1998-2005

Susy Carman, Christopher Olsen, Alexander Karasin, Beverly McEwen

The AHL uses antigen detection ELISA, IHC, FAT, PCR and virus isolation in eggs and in cell culture to identify influenza virus in swine herds. Using all virology tests, we have detected 182 virus-positive cases in the past 8 years, mostly from Ontario herds (Table 1). Identification of swine influenza viruses more than doubled in 2005, due to the outbreak of triple reassortant human-avian-swine H3N2 virus that swept across Canada in early 2005.

It is important to type swine influenza virus, since pigs can act as "mixing vessels" for swine, avian and human strains of 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 of all 8 gene segments. *(continued, p. 15)*

Influenza virus update *(continued)*

Table 1 lists the number of cases for each type of influenza virus recovered from 1998 through 2005 by the AHL and typed by either the AHL or the University of Wisconsin. In addition to classical H1N1 swine influenza viruses with all swine lineage genes, the AHL has recovered 2 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 virus, and 2 wholly avian H3N3 viruses. More recently, human-swine reassortant H1N1 viruses and human-swine reassortant H1N2 viruses have also been found in Ontario swine.

References

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Karasin AI, et al. Characterization of avian H3N3 and H1N1 influenza viruses isolated from pigs in Canada. J Clin Microbiol 2004;42:4349-4354.

Karasin AI, et al. Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada, 2003-2005. J Clin Microbiol 2006;44:1123-1126.

Table 1. Number of swine cases positive for influenza virus by any test, 1998-2005.

Year	Total cases for year	Total viruses isolated/yr	H1N1	H1N2	H3N2	H3N3	H4N6	Influenza A/ untyped
1998	14	13	12					1
1999	13	9	7		1		1	
2000	8	6	6					
2001	11	12	9		1	2		
2002	10	12	9					3
2003	11	8	7	1				
2004	32	14	11	1				2
2005	83	66	16		32			18
Totals	**182**	**140**	**77**	**2**	**34**	**2**	**1**	**24**

HORSES

Clostridium septicum fasciitis/myositis after intramuscular injection

Durda Slavic, Kimberly McGurrin, Maureen Anderson, Beverly McEwen

A 4 year old Standardbred mare was submitted to the AHL for necropsy. Clinical history indicated intramuscular injections in the neck over the course of 2 days. A local reaction at the injection sites spread subcutaneously over the whole neck. Ultrasound exam established the presence of subcutaneous emphysema and edema indicating possible clostridial infection. The mare deteriorated rapidly and was nonresponsive to treatment with debridement, penicillin, metronidazole and flunixin meglumine.

Gross post-mortem examination revealed marked subcutaneous edema extending from the muzzle to the proximal forelimbs. The edema was most severe in the fascia between all muscle layers of the right lateral neck and nuchal ligament. Marked peritracheal edema, submucosal edema of the epiglottis and vocal folds were also present.

Lung, spleen and muscle samples were submitted for bacteriology testing. Lung and spleen samples yielded no bacterial growth whereas a pure culture of gram-positive rods resembling *Clostridium* species was obtained from the muscle sample. Further genetic analysis of a partial 16S rDNA gene sequence revealed a 100% identity to *Clostridium septicum.*

C. septicum is a gram-positive anaerobic bacterium commonly found in soil and in feces of healthy animals and humans. **In horses, it is a main cause of malignant edema; a severe necrotizing soft tissue infection**. *C. septicum* infections are typically associated with intramuscular injections, although infections can also result from direct contamination of traumatic wounds. Regardless of the portal of entry, the condition is usually peracute with a high mortality rate. Because of the peracute nature of the condition, rapid diagnosis and treatment are very important. A successful outcome appears to be associated with high-dose intravenous antibiotic therapy and surgical debridement/fasciotomy of the affected area at the time of diagnosis. *AHL*

References

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Songer JG, Post KW. The genus *Clostridium*. In Veterinary Microbiology; Bacterial and Fungal Agents of Animal Disease. Elsevier Inc. 2005;261-282.

Horses, *continued*

ELISA replaces AGID for EIA testing

The agar gel immunodiffusion (AGID) test for equine infectious anemia (EIA) has replaced by the enzyme-linked immunosorbent assay (ELISA). The AHL has been accredited by CFIA to offer this test. As a routine, we will test Mondays, Wednesdays, and Fridays. Samples that arrive in the lab by 9 AM Friday will be tested and reported Friday. *AHL*

COMPANION ANIMALS

Comparison of synthetic depot ACTH with synthetic aqueous ACTH for adrenal stimulation testing in normal dogs

Tony Abrams-Ogg, Susan Atkinson, Dorothee Bienzle

In this study, we evaluated the blood cortisol response to both synthetic depot ACTH (Synacthen Depot) and aqueous ACTH (Cortrosyn) in 13 healthy dogs. These products contain the same ACTH molecule (cosyntropin/tetracosactrin/tetracosactide) but in Synacthen Depot, this molecule is complexed with zinc phosphate to delay absorption.

Seven dogs were assigned to a group receiving 0.25 mg of Cortrosyn IV, while 6 dogs received 1 mg of Synacthen Depot IM. Blood samples were taken prior to the injections, hourly over the next 8-hr period, and then at 24 hr post injection. One week later, the groupings were switched, and the dogs received the alternative ACTH formulation. Results were obtained for the mean ± SD serum cortisol concentrations (nmol/L) for a total of 13 samples (Fig. 1).

Overall, the Synacthen Depot resulted in higher and more prolonged increases in cortisol concentrations, but yielded a result comparable to Cortrosyn at 1 hr after injection.

Cortisol results 2 hr after injection of each product provided comparable results in some of the dogs. *AHL*

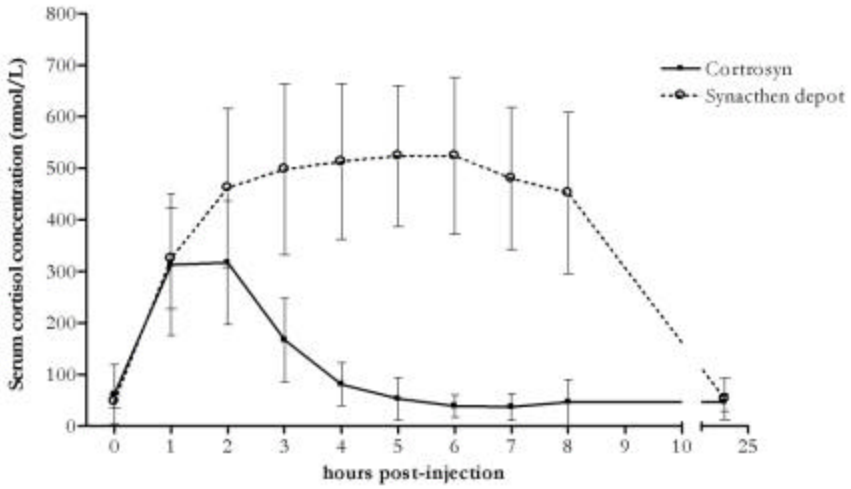

Figure 1. Serum cortisol response of dogs to Cortrosyn (0.25 mg IM; n = 7) and Synacthen Depot (1 mg IM; n = 6).

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