UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Dr. Mirjana Savic joins the AHL

Dr. Mirjana Savic (DVM '85, DVSc '90) began work August 2, 2005, as a regular full-time veterinary pathologist at AHL-Kemptville with Dr. Jan Shapiro. Mirjana has worked in veterinary labs in Fredericton NB and Truro NS, in industry, and as a private veterinary pathologist, and hence brings extensive experience with her. She was scheduled on duty in the Guelph lab for the first 3 weeks of August, and relocated to Ottawa with her family this summer.

Clinical pathology updates *Brent Hoff, Susan Atkinson*

New canine total T4 test available – new lower limits

In our experience, total T4 (TT4) >25 nmol/L in a suspect dog means that hypothyroidism is unlikely. Lower values (<20 nmol/L) are non-diagnostic in most patients and warrant further testing with other methods such as free T4 by dialysis and TSH. A TT4 <11 nmol/L may be considered diagnostic for hypothyroidism in some patients, but our previous TT4 kit was only accurate down to 13 nmol/L. The new Immulite Canine Total T4 kit will allow us to measure lower levels of TT4, with levels considered diagnostic for hypothyroidism in some canine patients (reference interval under review). Note that TT4 values may be affected by non-thyroidal illness and certain drugs and therefore can be falsely low.

New reference interval for canine serum calcium

Using serum from 52 healthy dogs, we have updated our canine serum calcium reference interval from 2.30 - 2.80 to **2.50 - 3.00 mmol/L**. This new RI appears on all AHL biochemistry reports.

Consolidated categories for cytology submissions

The following revised categories are posted in the Web version of the AHL Fee Schedule, available at http://www.uoguelph.ca/ahl/FeeSchedule/AHLFees.html

Test	Code	Lab	Fee	Comment	day	TAT
Cytology, fluids	cyto	clin path	40.00	bronchoalveolar lavage (BAL), cavity fluids, cerebrospinal fluid (CSF), synovial fluid - 2 mL fluid in EDTA tube + smears (maximum of 2 smears per site) - includes (as appropriate) cell count, protein concentration, Cytospin	M to F	same day
Cytology, smears	cytsm	clin path	30.00	staining and interpretation of smears [BAL, bone marrow, fine needle aspirates (FNA), impression, synovial, etc.], maximum of 10	M to F	same day
Histopathology/ cytology combination	histc	path / clin	49.00	combination of interpretation of cytology smears and histopathology confirmation on formalin-fixed tissue from the same lesion.	M to F	2-5 d

Submitting live animals

Josepha DeLay

Live animals submitted to the AHL for necropsy are given STAT priority. Animals are tranquilized immediately and are euthanized by the duty pathologist within 15-30 minutes of admission. Necropsy examination is carried out as soon as the animals are euthanized in order to maximize preservation of tissue detail and optimize diagnostic information for you and your client.

Because necropsy examination takes place within such a short time interval after admission, **it is imperative that referring veterinarians submit accurate histories either before the producer arrives at the lab with live animal submissions, or that the history accompanies the submission.** Your cooperation helps ensure that the diagnostic tests chosen at necropsy specifically target the herd problems recognized clinically. *AHL*

Bacterial counts of bedding or colostrum/milk

Jim Fairles, Durda Slavic

Although more research is needed to correlate bacterial counts of bedding and colostrum/milk with disease incidence, these parameters can be useful in management of disease outbreaks on individual farms. **For both bedding and colostrum/milk, relative bacterial counts may serve as an indication of the quality of sanitation measures on the farm**. This may in turn aid in the development of on-farm bedding/colostrum use and sanitation protocols.

The AHL has developed protocols for the collection and testing of these samples using Petri Film™. Please contact the AHL for the full protocol. For accurate bacterial counts, samples should be chilled as soon as they are collected and shipped on ice as soon as possible. Any delay in chilling the samples may lead to artificially high results.

Currently, we offer total aerobic and coliform bacterial counts, and both are reported as colony-forming units per milliliter (CFU/mL). Interpretation of these results should be approached with caution and used with all other clinical findings in the decision-making process.

Fees for these tests are:

Total bacterial count, Bedding - $15.00
Total bacterial count, Colostrum/milk - $13.50
Total aerobic and coliform count, Bedding - $17.80
Total aerobic and coliform count, Colostrum/milk - $16.20

References

McGuirk S, Collins M. Managing the production, storage and delivery of colostrums. Vet Clin Food Anim 2004;20:593-603.
Schukken Y, et al. Bedding cultures. OVMA Ann Conf. Jan 2005.

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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 laboratory-based animal disease events and disease trends.

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@lsd.uoguelph.ca*

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Contributors to this issue:
From the Animal Health Laboratory:
Susan Atkinson, BSc, MLT
Patricia Bell-Rogers, BSc, MSc
Susy Carman, DVM, Dip SA Med, PhD
Josepha DeLay, DVM, DVSc, Diplomate ACVP
Jim Fairles, DVM, MBA
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Brent Hoff, DVM, DVSc
Peter Lusis, DVM, MSc
Beverly McEwen, DVM, MSc, PhD, Diplomate ACVP
Jill Nicholson
Davor Ojkic, DVM, MSc, PhD
Jan Shapiro, DVM, Dip Path, Dip Eq Surg
Durda Slavic, DVM, MSc, PhD
Gary Thomson, DVM, MSc
Tony van Dreumel, DVM, MSc, Diplomate ACVP
Phil Watson, DVM
Sameh Youssef, BVSc, MVSc, DVSc, PhD, Diplomate ACVP

Other contributors:
Carlton Gyles DVM PhD, Kris Ruotsalo DVM DVSc Diplomate ACVP, Pathobiology, OVC

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.

Pathogenic vs. non-pathogenic *E. coli* isolates in animals with diarrhea

Durda Slavic, Carlton Gyles

Since *Escherichia coli* that are not capable of causing disease (non-pathogenic) are present in the intestine and feces of animals, it is essential to differentiate between pathogenic (disease-producing) and non-pathogenic *E. coli* isolates in the feces or intestines of animals with diarrhea. Several different types of pathogenic *E. coli* that may cause diarrhea are described, but by far the most common type is **enterotoxigenic *E. coli*** (ETEC). ETEC can be recognized by detecting structures on their surface, enterotoxins, or genes responsible for the surface structures and/or enterotoxins. Some of the surface structures (called O and K antigens) are not very specific for ETEC; others (fimbriae) are very specific but are sometimes not readily expressed when the bacteria are grown in the laboratory. The fimbriae that are very useful for detecting ETEC are F4 (formerly K88), F5 (formerly K99), F6 (formerly 987P), and F41.

Agglutination of isolated *E. coli* with antisera produced against the O and K antigens of ETEC (serotyping) is a relatively simple technique that is often used in the diagnostic laboratory for detecting *E. coli* that belong to the same serotype as ETEC. Typically, **pooled antisera** are used for this purpose. Pooled *E. coli* antisera used for detection of ETEC in our laboratory (pool 1N and 2N) contain a mixture of antibodies against O and K types of *E. coli* most frequently associated with diarrhea in pigs and/or calves. Although this approach is simple and relatively cheap, the results are sometimes misleading. False-positive results occur because the pooled antisera can cross-react with non-pathogenic *E. coli* isolates that have the same or related O and K antigens but do not produce the enterotoxins and/or fimbriae that are necessary for ETEC to cause diarrhea.

Agglutination tests may also be done with **monospecific antisera** that contain antibody against a single fimbrial antigen. Currently we are using antisera directed against F4 and F5 fimbrial antigens. These antisera are specific but fimbriae are not always produced by ETEC under laboratory conditions, and there are other fimbriae produced by some ETEC.

In contrast to agglutination tests that depend on the expression of the antigens to be detected, PCR-based methods detect the individual genes (DNA) that encode for these antigens and do not require that the antigen be expressed by the bacteria. In addition, when agglutination tests are done, usually up to 5 individual colonies are tested, whereas PCR can be done on a single colony or on a swipe from the whole plate, which increases the likelihood of detecting pathogenic *E. coli*. PCR methods are relatively simple and can give fast and accurate answers but they are more expensive. One could argue that the presence of genes without showing that they are expressed does not mean that these isolates are capable of causing diarrhea. However, **extensive testing of ETEC has shown that when genes for fimbriae and enterotoxins are present they are usually expressed.**

If ETEC is a concern, we recommend that you request PCR to obtain the most reliable results

Within our laboratory, a study with a limited number of isolates was done to determine the correlation between serotyping and genotyping (PCR) in ETEC isolates. The best correlation was obtained with F4 in pigs and F5 in calves. **Pool 1N performed very poorly and will therefore no longer be used for ETEC serotyping.** Pool 2N will be used, but the results need to be interpreted with caution. If ETEC is a concern, we recommend that you request PCR to obtain the most reliable results. This is especially important if an autogenous bacterin will be made.

Other types of pathogenic *E. coli* are sometimes implicated in diarrheal disease. These include **enteropathogenic *E. coli*** (EPEC) that may cause diarrhea in pigs, calves, and rabbits, and **enterohemorrhagic *E. coli*** (EHEC) that may cause diarrhea or bloody diarrhea in calves. We can also detect these pathogenic *E. coli* by PCR. *AHL*

References

DebRoy C, Maddox CW. Identification of virulence attributes of gastrointestinal *Escherichia coli* isolates of veterinary significance. Anim Health Res Rev 2001;2:129-140.

Gyles CL. ed. Diseases caused by *Escherichia coli*. In *Escherichia coli in Domestic Animals and Humans*. CAB International. 1994;73-333.

Songer JG, Post KW. The genera *Escherichia* and *Shigella*. In *Veterinary Microbiology; Bacterial and Fungal Agents of Animal Disease*. Elsevier Inc. 2005;113-120.

MIC testing now available

Durda Slavic

Minimal inhibitory concentration (MIC) testing for antimicrobials is now available at the AHL at a price of $25.00 per bacterial isolate. MIC testing is used to determine how much of each antimicrobial agent is required to inhibit bacterial growth. **This information is critical for clinical efficacy of the specific drug, because the dosage and the treatment regime should be designed in a way that the MIC is achieved in the targeted tissue and/or organ**. The general guidelines are:

- for *dose-dependent drugs*, the concentration in plasma should initially be 10X higher than MIC.
- for *time-dependent drugs*, the plasma concentration must be above the MIC for the whole dose interval.

Further information will be forthcoming from OMAFRA about clinical applications of MIC results. *AHL*

RUMINANTS

Bovine viral diarrhea virus genotyping at the AHL, 1998-2004

Susy Carman, Beverly McEwen

Over the last 7 years, the AHL has identified 1,111 strains of bovine viral diarrhea virus (BVDV) from various bovine specimens submitted for virus isolation or RT- PCR (Table 1).

The majority of the 1,050 BVDV isolates recovered in cell culture have been noncytopathic (79%), with only 21% being cytopathic.

Overall, using monoclonal antibodies or PCR genotyping, 485 (49%) strains were determined to be BVDV type 1, and 528 (51%) were BVDV type 2. *AHL*

Reference

Carman S, McEwen B. 2003 - Update on bovine viral diarrhea virus genotyping at the AHL. AHL Newsletter 2004;8:4.

Table 1. BVDV isolates by strain, biotype, and genotype, isolated at the AHL, 1998-2004

Year: / **BVDV type:**	**1998** n (%)[1]	**1999** n (%)	**2000** n (%)	**2001** n (%)	**2002** n (%)	**2003** n (%)	**2004** n (%)	**Total** n (%)
Total strains for each year (cell culture and RT-PCR)	199	166	122	170	142	168	144	1,111
Total isolated in cell culture	199	166	122	170	136	128	129	1,050
Total cytopathic	43 (*22*)	33 (*20*)	18 (*15*)	49 (*29*)	30 (*22*)	25 (*20*)	22 (*17*)	**220 (*21*)**
Total noncytopathic	156 (*78*)	133 (*80*)	104 (*85*)	121 (*71*)	106 (*78*)	103 (*80*)	107 (*83*)	**830 (*79*)**
BVDV type 1 cytopathic	26 (*13*)	23 (*14*)	7 (*6*)	42 (*35*)	20 (*27*)	7 (*14*)	13 (*42*)	138 (*28*)
noncytopathic	85 (*43*)	60 (*36*)	35 (*29*)	50 (*41*)	51 (*68*)	25 (*49*)	11 (*35*)	317 (*65*)
RT-PCR	nd	nd	nd	nd	4 (*5*)	19 (*37*)	7 (*23*)	30 (*6*)
Total type 1	**111 (*56*)**	**83 (*50*)**	**42 (*34*)**	**92 (*54*)**	**75 (*53*)**	**51 (*38*)**	**31 (*35*)**	**485 (*48*)**
BVDV type 2 cytopathic	15 (*8*)	10 (*6*)	11 (*9*)	7 (*1*)	10 (*15*)	16 (*19*)	6 (*11*)	75 (*14*)
noncytopathic	71 (*36*)	73 (*44*)	69 (*57*)	66 (*39*)	54 (*82*)	46 (*55*)	43 (*75*)	422 (*80*)
RT-PCR	nd	nd	nd	nd	2 (*3*)	21 (*25*)	8 (*14*)	31 (*6*)
Total type 2	**86 (*43*)**	**83 (*50*)**	**80 (*66*)**	**73 (*43*)**	**66 (*47*)**	**83 (*62*)**	**57 (*65*)**	**528 (*52*)**
BVDV type 1-2 mixture	2	0	0	1	0	0	0	3
BVDV untyped cytopathic	0	0	0	0	0	2 (*1*)	3 (*2*)	5 (*0.5*)
noncytopathic	0	0	0	4 (*2*)	1	32 (*19*)	53 (*37*)	90 (*8*)
Total untyped	**0**	**0**	**0**	**4 (*2*)**	**1**	**34 (*20*)**	**56 (*39*)**	**95 (*9*)**

[1] n (%) = number (*% of the subgroup for each year in italics*); nd = not done

Mycoplasma bovis and *Bovine viral diarrhea virus* in bovine respiratory disease pathology submissions, 2003-2005

Jan Shapiro, Phil Watson, Beverly McEwen, Jill Nicholson

Mycoplasma bovis was cultured from 58 pathology cases of bovine pneumonia submitted to the AHL in Guelph or Kemptville, from January 01, 2003 to May 31, 2005. Of these, 34 cases were from beef cattle and 24 were dairy cattle. *Mycoplasma bovis* was the only *Mycoplasma* spp. cultured in 35 % of the beef cattle cases and 54 % of dairy cattle cases; in the remainder of cases, *Mycoplasma arginini* and/or *Ureaplasma diversum* were also cultured.

Lungs, or lungs and other tissues, from the majority of the *M. bovis* positive cases were also tested for *Bovine viral diarrhea virus* (BVDV), using one or more of the following tests: virus isolation in cell culture, fluorescent antibody test, immunohistochemistry. **In both beef and dairy cattle with pneumonia from which *M. bovis* was cultured, 12.5% were also positive for BVDV.**

Pneumonia was accompanied by arthritis or polyarthritis in 2 dairy calf cases, and in 7 beef calf cases. One dairy calf and 1 beef calf also had otitis media. *AHL*

POULTRY

Update on H3N2 influenza A virus in Ontario poultry and swine

Susy Carman, Davor Ojkic

At the end of April 2005, the AHL laboratory began to recognize a new subtype of H3N2 influenza A virus in pigs in Ontario. Although not identified in Canada prior to 2005, this virus was shown to be similar to the Texas strain-like viruses present in US swine herds since 1998. These triple reassortant influenza viruses carry genes from human, pig and bird viruses. **This H3N2 influenza A virus has now been demonstrated in 25 swine herds in Ontario.** Many more have been infected. The hemagglutinin gene of the first 5 Ontario swine viruses and 3 similar viruses isolated at the AHL earlier in the year from swine herds in British Columbia, Alberta, and Manitoba were sequenced and compared. The nucleic acid sequence for all of these viruses was similar with 98.7% to 100% homology. For more information on swine influenza in Ontario see the OMAFRA website at http://www.gov.on.ca/OMAFRA/english/livestock/vet/disease_pre.html

As of July 21, there also have been **3 confirmed cases of H3N2 influenza A virus (Texas strain-like) in Ontario poultry**. The hemagglutinin gene sequence of the first Ontario H3N2 poultry virus was determined and compared to the recent H3N2 Canadian swine influenza A virus isolates. The poultry virus was 99.9% identical to a virus previously isolated from an Ontario swine herd. *AHL*

SWINE

Diagnosis of swine pneumonia

Peter Lusis

Swine pneumonias are often multifactorial and can be caused by viruses [PRRSV, *Porcine circovirus type 2* (PCV2), *Swine influenza virus* (SIV)], mycoplasmas, bacteria or any combination of the above. Causes of pneumonia can be difficult or impossible to differentiate in chronic cases.

- For **histopathology**, several lung sections from grossly affected, borderline, and 'normal' areas should be submitted. We often receive sections only from severely affected lung lobes, which can look very similar regardless of etiology. Immunohistochemistry (IHC) can be useful (PRRSV, PCV2, SIV) but influenza virus may only be present early in the disease, with 'false negative' IHC results.
- For **bacteriology**, 1 or 2 sections from grossly affected lobes, and bronchial lymph nodes, should be submitted.
- For **mycoplasmology**, culture for *Mycoplasma hyopneumoniae* is difficult, and cranioventral lobes should be submitted for PCR and/or FA.
- For **virology**, nasal swabs in viral transport medium (SIV, respiratory coronavirus isolation), nasal swabs in 0.5 mL saline (SIV antigen ELISA), and serum, lungs, thymus, tonsil and lymph node can be submitted for PCR and/or virus isolation. Serum can also be submitted for PRRSV and PCV2 ELISA's. *AHL*

Leptospiral abortion in swine

Peter Lusis, Gary Thomson, Josepha DeLay

Several near-term abortions occurred in a herd that had not been vaccinated for leptospirosis for several months. Gross and microscopic lesions were unremarkable or inconsistent and bacteriology, PRRSV and circovirus PCR tests were negative.

Leptospira interrogans serovar *bratislava* titers from fetal thoracic and/or pericardial fluid varied from 1:40 to 1:320. Detection of serovar *bratislava* antibodies in fetal or precolostral serum is an indication of fetal leptospirosis (but absence of detectable fetal antibodies does not exclude a diagnosis of leptospirosis). **Fetal leptospirosis serology is therefore a valuable test for swine abortion diagnosis**. *AHL*

Reference

Bolin CA, et al. Reproductive failure associated with *Leptospira interrogans* serovar bratislava infection of swine. J Vet Diagn Invest 1991;3:152-154.

Porcine circovirus type 2-associated disease is increasing

Josepha Delay, Beverly McEwen, Susy Carman, Tony van Dreumel, Jim Fairles

Porcine circovirus type 2 (PCV2)-associated disease has increased markedly in 2005 compared to the previous 7 years (Fig. 1). Although many of the cases have had other pathogens identified (e.g., PRRSV, *Streptococcus suis*), many cases have had PCV2 identified as the only agent. Most necropsy cases of PCV2-associated disease seen at the AHL during 2005 have some of the typical lesions of this disease, including poor body condition, firm lungs that fail to collapse (implying interstitial pneumonia), and multiple pale enlarged lymph nodes.

Several additional lesions are 'new' to Ontario pigs with PCV2 infections:

- Prominent pulmonary interlobular edema has been observed in many pigs. Histologically, interlobular septa are widely expanded by edema, and alveoli are flooded by proteinaceous edema fluid, but infiltration of mononuclear inflammatory cells in alveolar septa is generally much less pronounced than in traditional PCV2 cases.
- Thickening of the walls of ileum and colon, sometimes accompanied by mucosal erosion or ulceration and reminiscent of porcine proliferative enteritis due to *Lawsonia intracellularis*, has been identified in many pigs with diarrhea. Histologically, these animals have granulomatous enteritis and colitis, with extensive infiltration of histiocytes and fewer lymphocytes throughout the lamina propria.
- In lymph node, histologic lesions of lymphoid depletion and histiocytic infiltration are more severe and extensive than previously seen, with large numbers of typical circoviral inclusions in histiocytes and multinucleated giant cells. In recent cases, spleen and tonsil are also frequently affected, and sometimes have evidence of acute lymphoid necrosis.

Typically, all manifestations of PCV2-associated disease in recent cases have much larger viral antigen loads, as demonstrated by immunohistochemistry, than we have seen routinely in cases prior to 2005.

Concurrent with these changes in the pathology of PCV2-associated disease, cases of suspected PCV2-related vasculitis and immune-complex disease (porcine dermatopathy and nephropathy syndrome) have increased. Lesions commonly involve kidney, lymph node, and spleen, with variable lung involvement, although cutaneous lesions have not been present in affected animals. Vasculitis (including glomerulonephritis) is present consistently, sometimes with obvious splenic infarcts, and these lesions make it necessary to consider classical swine fever as a differential diagnosis. PCV2 antigen is rarely evident in association with vascular lesions, as expected in immune-complex disease, although variable amounts of antigen are present in other tissues such as lung in some cases. We have also seen several cases of pigs with clinical neurologic disease in which PCV2 antigen was demonstrated in association with endothelial or inflammatory cells in brain, as well as in other tissues.

Compared to 1998, a swine submission to the AHL in 2005 is now 14 times more likely to have PCV2-associated disease on histopathology. PCV2 PCR-RFLP typing for all PCR testing requests shows that a significant change from RFLP type 422 to type 321 also occurred in 2005 (Fig. 2). These changes in RFLP typing are the result of a consistent change in gene sequence, recognized by two restriction enzymes. *AHL*

Reference

van Dreumel T, Josephson G, Lusis P. *Porcine circovirus-2* associated conditions in pigs. AHL Newsletter, 2005;9(1):5.

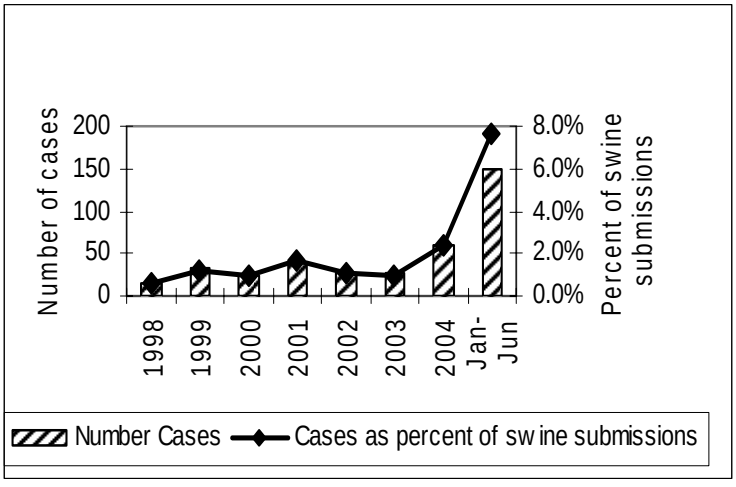

Figure 1. Number of PCV2 pathology cases and percentage of PCV2 pathology cases of total swine submissions.

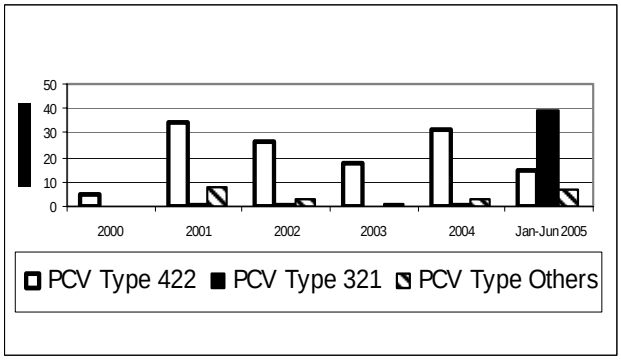

Figure 2. PCR-RFLP typing of PCV2.

IDEXX H3N2 swine influenza virus antibody ELISA now available at AHL

Susy Carman, Beverly McEwen, Jim Fairles

The IDEXX swine influenza virus (SIV) H3N2 antibody ELISA - approved by the USDA and available at the AHL as of June 2005 - is intended to monitor swine herds for antibody to all strains of H3N2 SIV, including the H3N2 triple reassortant viruses recently introduced into Ontario swine herds. The diagnostic sensitivity of the H3N2 ELISA is 96.1% (91.1,99.7), which is greater than the H3N2 HI test, and the diagnostic specificity of the H3N2 ELISA is 89.0% (80.7,95.9), which is similar to the H3N2 HI test.

The fee is **$6.00 per test**. Please request this H3N2 antibody ELISA by writing directly on the AHL swine submission form, as there is no check-off box on the current form for this new test.

Results of the IDEXX H3N2 ELISA are reported similarly to those of the IDEXX PRRSV and H1N1 SIV antibody ELISA's, i.e., as sample-to-positive ratio (S/P), titer group, and mean S/P ratio.

However, **the H3N2 S/P results are interpreted slightly differently from the other 2 ELISA's**:

- S/P ratios <0.30 are **negative** for antibody to SIV H3N2.
- S/P ratios ≥0.30 and <0.40 are **suspicious** for antibody to SIV H3N2.
- S/P ratios ≥0.40 are **positive** for antibody to SIV H3N2.
- Positive results indicate exposure to SIV H3N2 or to vaccines derived from this virus. This test is not intended to distinguish between virus subtype-specific exposures.
- Low-level cross-reactivity of this test with antibodies to other subtypes of swine influenza virus, such as H1N1, may occur.

For more information, please contact:

Dr. Susy Carman at 519-824-4120 ext 54551, scarman@lsd.uoguelph.ca or
Dr. Jim Fairles at 519-824-4120 ext 54611, jfairles@lsd.uoguelph.ca *AHL*

HORSES

Diagnosis of equine Cushing's disease

Grant Maxie, Kris Ruotsalo, Brent Hoff, Susan Atkinson

Equine Cushing's disease (**pituitary pars intermedia dysfunction**, PPID) may occur as the result of pituitary pars intermedia hypertrophy, hyperplasia, or adenoma. PPID is a frequent diagnosis in aged horses. Affected individuals are usually at least 7-yrs-old, with an average age at presentation of 20 yr. After an insidious onset, the most common clinical signs are hirsutism, weight loss, lethargy, laminitis, polyuria and polydipsia, and hyperhidrosis. Affected horses are not necessarily hyperglycemic. Two of the major complications of PPID are diabetes mellitus and laminitis. In addition to determination of serum glucose, insulin, and endogenous ACTH concentrations, the clinical diagnosis of Cushing's disease can be supported by means of an **overnight dexamethasone suppression test** - blood samples taken for serum cortisol analysis before and 20 hrs after IM administration of dexamethasone at 40 µg/kg BW at 1700 hrs. Healthy horses should have a serum cortisol concentration of <27.6 nmol/L at 20 hrs post-dexamethasone (equine cortisol reference interval is 50-640 nmol/L).

Note that antemortem diagnosis of PPID in horses is problematic. In the fall, seasonal increases in pars intermedia function may lead to a *false-positive* diagnosis. A *false-negative* diagnosis is possible in early PPID. As with all laboratory testing, results must be interpreted in light of history and clinical signs.

We have replaced our current equine endocrine panels, including "Cushing's syndrome (T4, cortisol, insulin, glucose)", with the panels below. *AHL*

References

McFarlane D. Obesity and pituitary pars intermedia dysfunction in aging horses. Proc CVMA Convention 2005:237-239.

van der Kolk H. Diseases of the pituitary gland, including hyperadrenocorticism. In *Metabolic and Endocrine Problems of the Horse*, ed. Watson T. Toronto: WB Saunders. 1998:41-59.

Test	Code	Lab	Fee	Comment	day	TAT
Equine thyroid panel	eep1	clin path	40.00	TT4, fT4D - 1 mL serum	M to F	7 d
Equine Cushing's panel 1	eep2	clin path	90.00	fT4D, ACTH, glucose - 1 mL serum + 1 mL EDTA plasma frozen in a plastic tube (ship Mon-Thurs)	M to F	14 d
Equine Cushing's panel 2	eep3	clin path	95.00	insulin, ACTH, glucose - 1 mL serum + 1 mL EDTA plasma frozen in a plastic tube (ship Mon-Thurs)	M to F	14 d
Equine dexamethasone suppression test	corde	clin path	32.00	0.5 mL serum at 0 hrs and 20 hrs (next day) after 40 ug/kg BW dexamethasone IM at 1700 hrs	M to F	1 d

COMPANION ANIMALS

A cluster of *Canid herpesvirus 1* infections

Sameh Youssef, Tony van Dreumel

Between November 2004 and February 2005, **we diagnosed canine herpesvirus infection in 5 unrelated litters of puppies** at the AHL in Guelph. These cases represent ~30% of the cases diagnosed from 1998 to July 2005. Affected puppies ranged in age from 1-5 weeks with a clinical presentation of dullness and dyspnea and a mortality rate of 20-50% in affected litters. There was no breed, sex, or geographical area predilection. Histology revealed multifocal necrohemorrhagic areas typical of alphaherpesvirus infection in kidneys, liver, adrenal glands and lung, with the presence of characteristic intranuclear inclusions. Mild nonsuppurative encephalitis and lymphoplasmacytic interstitial pneumonia were also present occasionally.

Canine herpesvirus disease normally affects puppies <1 week of age and is caused by *Canid herpesvirus 1*, an alphaherpesvirus belonging to the family *Herpesviridae*. Infection of puppies takes place by contact with infectious oral, nasal or vaginal secretions during or shortly after parturition; in utero infection is also reported. The disease seldom occurs in puppies >1 week of age, however the average age of the current cases was 18 days, with one submission of 5-week-old puppies. **This unusual presentation may reflect waning immunity due to associated stress** (e.g., cold, concurrent disease, immunosuppressive therapy). No significant concurrent disease was identified in these submissions. As vaccination for this disease is not practiced, recommendations include reducing stress (e.g., housing newborn puppies in a warm environment, paying particular attention to insulation of the floor of the whelping box) and minimizing contact between pregnant bitches and other dogs. *AHL*

Reference

Carter GR, et al. A Concise Review of Veterinary Virology. IVIS. 2004 http://www.ivis.org/advances/Carter/toc.asp

Canine parvovirus 2 antigen ELISA: Interpretation of test results

Susy Carman, Josepha DeLay

Fecal testing for *Canine parvovirus 2* (CPV-2) using an antigen detection ELISA is commonly used both at the AHL and as an in-clinic test for dogs with clinical signs consistent with parvoviral enteritis. Confusion frequently arises when CPV-2 antigen is not detected by antigen ELISA in feces from animals diagnosed clinically as having classic CPV-2 associated disease and/or pathognomic histologic lesions at necropsy.

The most likely reason for a false-negative fecal antigen ELISA is the outflow of blood and CPV-2 serum antibody into the gut following the loss of the epithelial cell lining of the gut at the time of clinical disease at about 6-9 days post-infection. Antibody to CPV-2 develops as soon as day 3 following infection, and can be present in sufficient concentration to bind and neutralize virus within the intestinal lumen producing false-negative test results using both the antigen detection ELISA and virus isolation. Because virus is hidden inside cells away from neutralizing antibody following fixation in either acetone or formalin, immunofluorescence and immunohistochemistry tests for tissues may be positive for longer time periods (about 2 extra days).

This reinforces the importance of considering clinical signs in conjunction with results of laboratory testing when diagnosing infectious diseases. **A positive fecal antigen ELISA result is helpful to confirm CPV-2 infection; however, a negative result does not exclude CPV-2 if clinical signs are consistent with parvoviral enteritis.** *AHL*

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