UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

LAB REPORTS

Transportation of live animals to the laboratory

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Live mammals and birds, especially if untreated and in early stages of disease (neonatal diarrhea, egg production drop, etc.), are very useful, and sometimes essential (e.g., TGE), for laboratory diagnosis. However, we occasionally receive live animals that have been transported inhumanely, e.g., several live piglets or chickens crowded together in a cardboard box or feed bag, with several hours elapsed before delivery on a hot (or a cold) day; recently a live goat kid was submitted in a tied-off plastic grocery bag!! Some of these animals have died in transit, presumably due to heatstroke, suffocation, injury, or other stress.

If intercepted by the police or Humane Society, transporters of animals under such conditions and/or their owners would no doubt be charged with cruelty to animals - just think of the media coverage when a dog is left unattended in a car on a hot summer day! According to federal **Bill C-15B, animal cruelty** includes "anyone who **willfully or recklessly** a) **causes or**, being the owner, **permits** to be caused, **unnecessary pain, suffering or injury** to an animal, ….."

Providing feed and water in most shipments to us should not be necessary if i) **transport time is as short as possible,** ii) **animals are not overcrowded,** and iii) **there is adequate ventilation and protection from excessive heat or cold.** We euthanize live animals as soon as possible after arrival at the laboratory to minimize stress.

After blood and other samples are collected, most **large animals** (>100 kg) should be euthanized before transport and submitted to the lab as soon as possible after death. **Please discuss with a pathologist** if you feel that submission of a live large animal is preferable.

It is our duty as veterinarians to prevent inhumane treatment of animals. We will report serious or repeat offenders to the SPCA or the Ontario Farm Animal Council.

Bile acid methodology change

Susan Atkinson *satkinso@lsd.uoguelph.ca*

We have changed the bile acid methodology from a manual method to an automated procedure on the Hitachi 911 analyzer, which is more efficient, less subject to interferences by lipemia, icterus and hemolysis, and has a shorter turnaround time. It also has the advantage of requiring less sample volume (0.2 mL serum or plasma) than the "old" method (0.5 mL serum).

Our comparison studies have shown good correlation at bile acid concentrations typical for healthy animals and those with mild elevations using the "old" method.

Marked increases by the "old" method are less dramatic with the automated new method. These features are deemed acceptable. **New reference intervals** have been developed in the AHL Clinical Pathology laboratory, and are similar to those determined at other institutions.

AHL bile acid reference intervals:

Canine (n=34)	0-6 µmol/L	fasting
	0-20 µmol/L	post-prandial
Feline (n=15)	0-3 µmol/L	fasting
	0-20 µmol/L	post-prandial
Equine (n=11)	0-6 µmol/L	
(preliminary results)		

Clostridium perfringens genotyping, a valuable component of the diagnosis!

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Clostridium perfringens is the most important cause of clostridial enteric disease in domestic animals. These infections are common presentations at the AHL diagnostic lab. A diagnosis of *C. perfringens* disease can be achieved by gathering key elements: history and evaluation of clinical signs, gross and microscopic lesions, bacteriological culture of specimens with a clostridial cell count, and genotyping of *C. perfringens* by multiplex polymerase chain reaction (PCR) to determine the type based on toxin genes. These criteria form a continuum of diagnoses. This comprehensive approach is useful in establishing diagnoses of clostridial disease and will allow a better understanding of the prevalence of *C. perfringens* disease in Ontario with an easier evaluation of prophylaxis, therapy and control measures.

C. perfringens strains can be separated based on four major toxins: alpha, beta, epsilon and iota. **Type A** is defined as strains possessing the gene for alpha toxin**, type B** as strains with alpha, beta, and epsilon toxin genes, **type C** as strains with the genes for alpha and beta toxins, **type D** as strains with the genes for alpha and epsilon toxins, **type E** as strains having the genes for alpha and iota toxins. In addition, these types may or may not have the cpe-toxin gene or the beta-2-toxin gene. An example of a genotyping gel from the analytical method is presented in Figure 1. The *C. perfringens* type as well as the genes encoding the different toxins will appear on your AHL genotyping report (Table 1).

C. perfringens infections take many forms in many different hosts:

- **Type A** strains are widespread in the intestinal tracts of animals and in the environment. They can cause soft tissue diseases (wound contamination, cellulitis/ myonecrosis, and gas gangrene) and enteric diseases (food poisoning, enterotoxemia in lambs and cattle, abomasitis/abomasal ulceration, necrotic enteritis in fowl, necrotizing enterocolitis in piglets, equine colitis, and canine hemorrhagic gastroenteritis).
- **Type B** strains are relatively rare in Canada, and cause enteric diseases in newborn lambs (enterotoxemia with enteritis and extensive hemorrhage and ulceration of the small intestine). The primary sign is usually sudden death in peracute cases. Chronic cases (a disease called 'pine') in older lambs result in chronic abdominal pain without diarrhea. Type B can also be associated with hemorrhagic enteritis in goats, calves, and foals.
- **Type C** strains cause enteric diseases in pigs, cattle, sheep, horses, chickens, humans, and dogs. Peracute infection may affect piglets 1 to 2 days of age where diarrhea, dysentery and necrotic debris can be observed. Piglets infected at 1 to 2 weeks of age will have non-bloody, yellow diarrhea and necrosis of the jejunal mucosa. A similar manifestation of the disease occurs in neonatal calves, lambs, and goats. Nervous signs may also occur. *(continued on page 27)*

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

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- **Type D** strains cause enterotoxemia, also known as **sudden death or overeating or pulpy kidney disease**, in sheep of all ages except newborns. In these cases, a true toxemia results with usually no evidence of enteritis. The epsilon toxin acts on the central nervous system and causes sudden death. Some animals will survive long enough to develop neurological signs - the result of focal symmetrical encephalomalacia *(see FSE article, page 30, this issue)*. Type D enterotoxemia is also seen in calves, goats, and (rarely) in adult cattle, deer, or horses. The disease in calves is similar to the disease in sheep. However, hemorrhagic enterocolitis without the pulpy kidney lesion is a common finding in goats.
- **Type E** strains are uncommon causes of enterotoxemia of lambs, calves, and rabbits.

The **cpe gene** encodes for an enterotoxin that can be produced by types A, B, C, D or E. If present, these types are also named enterotoxigenic strains. This enterotoxin is a pore-forming toxin that causes cell lysis. The ***beta-2* (ß2) gene** has been recently described and is strongly related to enteritis in piglets. Nearly 100% of type A isolates from normal piglets are beta-2 negative, whereas 90% of those from piglets with enteritis are beta-2 positive. This suggests that the beta-2 toxin may play a key role in the pathogenesis of the disease. An *in vivo* synergistic role of the alpha and beta-2 toxins in the production of necrotic and hemorrhagic lesions of the small intestine in cases of bovine enterotoxemia has also been recently proposed.

Genotyping is a valuable component of the diagnosis. It provides data to determine whether a significant association exists between a specific gene and a specific *C. perfringens* disease in a particular host. Improving our Ontario diagnostic capabilities regarding *C. perfringens* diseases will contribute to a better understanding of the risk factors that contribute to the development of disease by this opportunistic agent. Eventually, this information will allow us to better prevent and control these infections.

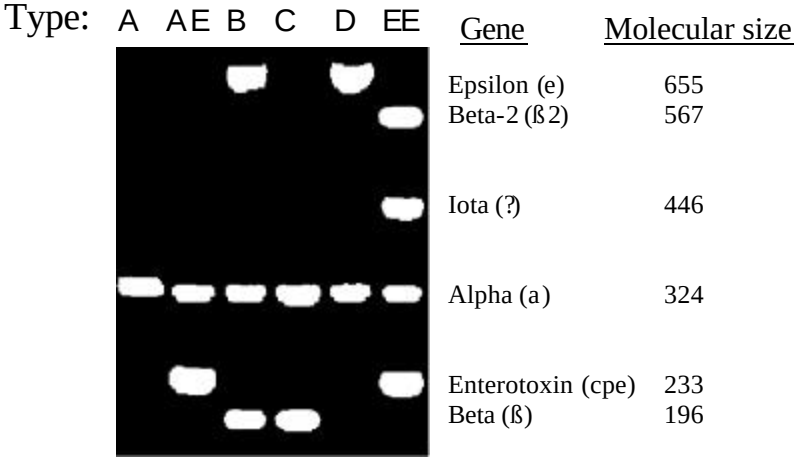
References

1. Manteca C, Daube G, Jauniaux T, et al. A role for the *Clostridium perfringens* beta-2 toxin in bovine enterotoxaemia. Vet Microbiol 2002; 86: 191-202.
2. Garmory HS, Chanter N, French NP, et al. Occurrence of *Clostridium perfringens* ß2-toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemiol Infect 2000; 124: 61-67 .
3. Songer JG. Clostridial enteric diseases of domestic animals. Clin Microbiol Rev 1996; 9: 216-234.

Table 1. *Clostridium perfringens* genotyping based on the major toxin detected

	Major toxin present			
Type	Alpha(a)	Beta(ß)	Epsilon(e)	Iota(?)
A	+	-	-	-
B	+	+	+	-
C	+	+	-	-
D	+	-	+	-
E	+	-	-	+

Figure 1. Typical multiplex PCR gel showing the *Clostridium perfringens* strain type, responsible gene, and molecular size (base pairs) of PCR products.



Additional immunohistochemistry tests available at the AHL

Josepha DeLay *jdelay@lsd.uoguelph.ca*

Development of new immunohistochemistry (IHC) tests for the detection of infectious agents and tumor markers continues at the AHL. Tests are now available to detect the following viral antigens in formalin-fixed tissue sections: **transmissible gastroenteritis virus (TGEV), bovine viral diarrhea virus (BVDV),** and ***Toxoplasma gondii***. Tumor markers currently available are **lymphocyte markers CD79a and CD3, melan-A, vimentin, and S-100**. Tests under development include those to detect bovine respiratory syncytial virus (BRSV), *Chlamydia* spp, and *Leptospira* spp, and these tests should be available within 1 to 2 months. Immunohistochemical tests are also being used at the AHL in surveillance programs for **bovine spongiform encephalopathy (BSE)** and **cervid chronic wasting disease (CWD).**

> In most cases, the recommendation to proceed with IHC on an individual case will be based on histologic lesions

IHC for identification of infectious agents complements other tests such as virus isolation and fluorescent antibody (FA) testing. It is a useful adjunct in cases with suspicious histologic lesions but inconclusive results from other testing procedures, or in situations where only formalin-fixed tissue is available for further evaluation. IHC for TGE virus is especially useful for mail-in cases where this disease is suspected, as samples submitted by courier cannot be maintained in frozen condition necessary for FA testing. IHC for tumor markers can aid in determining the cell line of origin of poorly differentiated and other neoplasms, and can provide important prognostic information.

In most cases, the recommendation to proceed with IHC on an individual case will be based on histologic lesions, and the pathologist will communicate this information to the referring veterinarian prior to ordering the test.

The price for IHC involving food-producing animals is $20 per case. For non-food-producing animals, the charge for one antigen test on a single tissue block is $44; additional tissue blocks or antigens tested from the same case are $21 each.

Updates will follow in future AHL newsletters as new IHC tests become available, and current tests will also be listed on our website. For additional information, please contact Dr. Josepha DeLay at the AHL, phone (519) 824-4120 ext. 4576.

Neospora caninum cELISA replacement

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We are now offering a competitive-inhibition (c) ELISA for the detection of serum antibodies (Abs) against *N. caninum.* The IDEXX *N. caninum* ELISA is no longer available. The cELISA, manufactured by VMRD Inc. of Pullman, WA, is based on a monoclonal antibody (mAb) against an immunodominant epitope of *N. caninum* and **can be used to test serum samples from both cattle and dogs**. If Abs to *N. caninum* are present in the sample, they will inhibit the binding of the horseradish peroxidase conjugated mAb to the ELISA microplate that was coated with *N. caninum* antigen. Since the binding of the mAb is detected by addition of an enzyme substrate and color development, weak color caused by inhibition of the mAb binding indicates the presence of Abs to *N. caninum* in the sample.

Samples having less than 30% inhibition are negative, while samples having 30% or greater inhibition are positive. The data obtained from the manufacturer states that diagnostic sensitivity was 97.6% and specificity 98.6% when the assay was validated on a set of 184 cow sera defined by fetal histopathology and *N. caninum* immunohistochemistry. When the assay was validated on a set of 192 canine samples defined by IFA, diagnostic sensitivity was 91.4% and specificity was 99.4%.

The test is set up weekly, or more frequently, depending on demand. The fee is $7.00 for food-producing animals and $15.00 for dogs.

Reference

Baszler TV, Adams S, Vander-Schalie J, Mathison B, Kostovic M. Validation of a commercially available monoclonal antibody-based competitive-inhibition ELISA for detection of serum antibodies to *N. caninum* in cattle. J Clin Microbiol 2001; 39: 3851-3857.

Further restrictions on deadstock - no sulfonamide residues

From Brief Communication, OABP/OASP/Veterinary Science, OMAF—July, 2002

As of September 15, 2002, dead animals destined for rendering at Maple Leaf Foods (Rothsay) must be certified free of any sulfonamide compound, not just sulfamethazine as at present.

AHL Lab Reports

CATTLE

Ruptured abdominal arteries in dairy cattle

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We have recently seen several cases of fatal internal hemorrhage from various abdominal arteries in Eastern Ontario dairy cattle - in one herd, at least 3 mature cattle died from this over a period of several weeks. We also occasionally see fatal hemorrhage from ruptured uterine arteries in periparturient dairy cattle without evidence of uterine torsion at the time of necropsy. Similar findings have been reported from upstate New York and Quebec.

Copper deficiency has been suspected, but not confirmed, in cases of uterine arterial rupture in mares. In dairy cattle, arterial ruptures may have been corrected by copper supplementation in some herds in New York State (1), but liver copper levels were normal or above normal in the few cases that we examined. Marfan's disease has been reported in one group of genetically related cattle in the western US, but this is characterized by rupture of thoracic, not abdominal, arteries.

We are attempting to trace common possible genetic and other factors among affected cattle but this is difficult in non-purebred herds. **Please notify us if you see any cases of ruptured abdominal arteries in dairy cattle - we would be interested in investigating these cases, especially in herds with more than one cow involved.**

Reference

1. Dr Brad Njaa, Cornell University, personal communication.

A case of inorganic arsenic toxicosis in a beef herd

Brent Hoff, Ted Delange, Meredith Faires *bhoff@lsd.uoguelph.ca*

During a 7-week period, 5 calves from a 170-head beef herd developed clinical signs of weakness, ataxia, depression, dehydration, diarrhea, and loss of appetite. Four of the affected animals died.

Hematological examination revealed leukopenia, neutropenia, and hemoconcentration. A bovine serum chemistry profile revealed abnormal elevations in urea, creatinine, creatine kinase, glutamic dehydrogenase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, and total and conjugated bilirubin. These results suggested hepatopathy and severe renal dysfunction.

Histological changes of formalin-fixed tissue included focal areas of necrosis in the periportal areas of the liver, mild nephrosis, necrotizing hemorrhagic enteritis, and marked congestion of the red pulp of the spleen.

Following the death of the fourth calf, a dumpsite was discovered in the pasture where the cattle grazed. **Within the debris was an open metal container half-filled with white powder that had appeared to be ingested by the animals**. At that time, the owner of the pasture tentatively identified the powder as an old potato dust that could have been used at a much earlier time. Frozen samples of liver and rumen content from the fourth calf as well as the white powder were sent for toxicological analysis at the AHL. Using x-ray diffraction and inductively coupled plasma (ICP) spectroscopy, **the white powder was found to contain 70% arsenic trioxide (As_2O_3), with the liver containing 353 ppm and the rumen content containing 1200 ppm As_2O_3.**

Clinical signs of **acute arsenic toxicosis** include prostration, diarrhea, trembling, staggering, extreme weakness, dehydration, with high morbidity and mortality over a 2-3 day period. Animals suffering from **subacute toxicosis** may display depression, anorexia, dehydration, polydipsia, anuria, weakness, trembling, hypothermia, and death. Percutaneous absorption causes capillary dilation and degeneration leading to blistering, edema, and dry, cracked skin.

Inorganic arsenic poisoning is no longer common due to the decreased use of herbicides and insecticides containing arsenic. However, many sources are still available, including insecticides, defoliants, paints, baits and outmoded medications. Diseases frequently confused with arsenic poisoning, especially in ruminants, include bacterial and viral infections, urea toxicosis, organophosphate toxicosis, other heavy metals, as well as a variety of toxic plants.

Reference Osweiler, GD, Carson, TL, Buck, WB, Van Gelder, GA. Clinical and Diagnostic Veterinary Toxicology. 3rd ed., 1985, Kendall/Hunt Publishing Company.

SMALL RUMINANTS

Focal symmetrical encephalomalacia in two lambs

Murray Hazlett, Scott Reid *mhazlett@lsd.uoguelph.ca*

In May, two 4-month-old lambs were submitted for examination and necropsy to the AHL in Guelph. These lambs had been showing severe neurologic disease (profound stupor, staggering, nystagmus) as well as diarrhea for two days. At arrival, one was moribund, and one could still stand but was very reluctant to move. The lambs had been fed creep feed since February and had been on pas ture for the past 2 weeks. They had both been treated with penicillin by the owner with no improvement. Although the ewes had been vaccinated for clostridial diseases, the lambs were not vaccinated.

At necropsy, there were no significant gross internal findings except for multifocal hemorrhage in the cerebral caudate nucleus of one lamb (Fig 1). Both lambs had grassy content in rumens, scant small intestinal content, and hard-formed fecal pellets in colon. Microscopically, areas of necrosis were seen, sometimes with hemorrhage, in the basal nuclei/internal capsule region and cerebellar peduncles. An area of necrosis was also seen in cerebellar folia of one lamb. *Clostridium perfringens* could not be recovered from these animals, likely because of the antibiotic treatment.

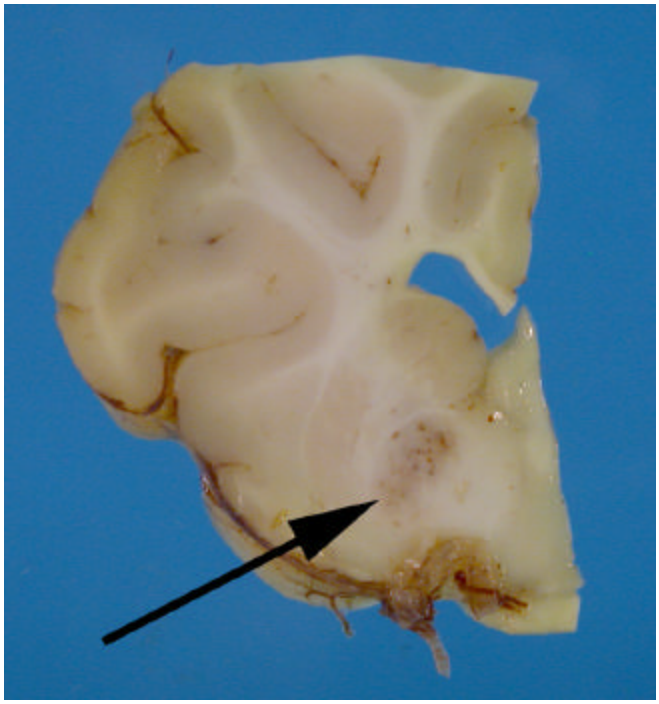

Figure 1. Hemorrhage in the caudate nucleus of a lamb with focal symmetrical encephalomalacia.

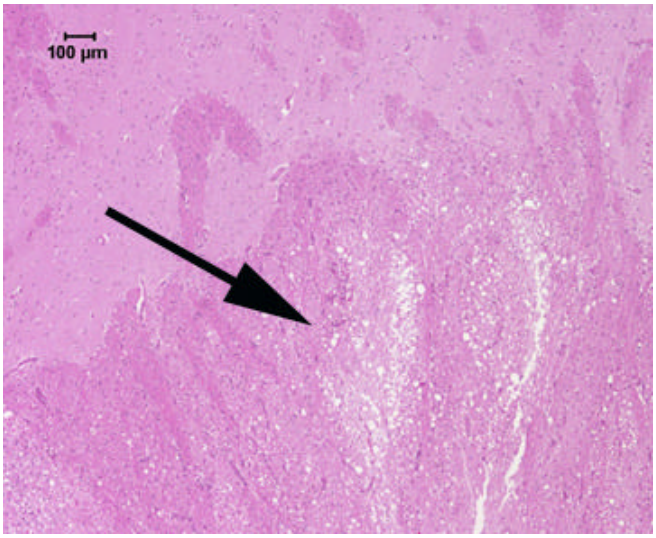

Figure 2. Photomicrograph showing malacia (arrow) in the caudate nucleus of a lamb with focal symmetrical encephalomalacia.

Most lambs with *Clostridium perfringens* type D enterotoxemia have a very short clinical course and are often found dead with no specific gross or histologic changes. Older animals may survive longer (several days), showing nervous signs and developing lesions of focal symmetrical encephalomalacia (FSE). **FSE is caused by the epsilon toxin of *C. perfringens* type D**. Neurologic signs include head pressing, visual impairment, and loss of balance. Interestingly, goats do not seem to develop nervous signs (1).

Subsequent to the laboratory diagnosis, the lambs were vaccinated for *C. perfringens* type D. No further losses occurred.

Reference

1. Barker IK, van Dreumel AA, Palmer N. In, Pathology of Domestic Animals, ed 4, vol 2 p 241.

POULTRY

Infectious bursal disease virus - diagnostic update

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

In spite of widespread vaccination, infectious bursal disease virus (IBDV) continues to present a problem for the Ontario broiler industry. In the US, numerous serologic "variant" IBDV's have been described that may escape the immunity induced by vaccines based on so-called "classical" IBDV strains. In order to enhance our capabilities in detection and characterization of field IBDV's, the AHL has introduced several new diagnostic methods briefly described below.

1. IDEXX IBDxr ELISA. We now offer an improved ELISA that facilitates enhanced detection of serum antibodies to variant IBDV strains and better correlates to virus neutralization compared to classical IBDV ELISA. IBDxr provides an increased range as well as a more accurate evaluation of vaccination protocols and/or disease status. Please note that the baseline IBDV titers will shift and flock antibody profiles may need to be re-assessed when switching from classical to IBDxr ELISA. The price of the IBDxr test is $2.75/ sample.

Infectious bursal disease virus (IBDV) continues to present a problem for the Ontario broiler industry.

2. IBDV reverse-transcriptase polymerase chain reaction (RT-PCR). Molecular testing is conducted on fresh or frozen bursal tissue**.** We have adopted an RT-PCR assay for testing of bursal tissues for the presence of IBDV genetic material (1). Upon request, IBDV-positive RT-PCR samples can be genotyped by restriction fragment length polymorphism (RFLP) analysis and/or Vp2 gene sequencing. The price for RT-PCR is $16.00/sample.

3. IBDV RFLP analysis. Genetic differences believed to be responsible for the antigenic variation of IBDV field strains may be detected by RFLP analysis. RT-PCR positive samples can be divided into different molecular groups based on their RFLP profiles. RFLP analysis can differentiate between vaccine and field viruses. In North America, IBDV strains are currently classified into six molecular groups: variant viruses belong to groups 1 and 2, classical viruses are in groups 3, 4 and 6, while viruses in group 5 are related to the Lukert strain (2). IBDV field strains that belong to the same group are likely related antigenically. The price for RFLP analysis is $32.00/sample.

4. IBDV Vp2 gene sequencing/phylogenetic analysis. Positive RT-PCR samples can also be analyzed by Vp2 gene sequencing, which is especially recommended when a "new" IBDV profile is discovered by RFLP. The advantage of IBDV Vp2 gene sequencing is that it can more precisely evaluate the genetic background of a field IBDV strains than is possible with the RFLP analysis. Genotyping by Vp2 gene sequencing/phylogenetic analysis combines RT-PCR, sequencing and sequence analysis into the most powerful IBDV diagnostic technique that is currently available. This test can also differentiate between vaccine and field strains and can be used to quickly compare new field IBDV strains with all previously characterized viruses. The price for IBDV VP2 gene sequencing is $128.00/sample.

Histopathology. Histologic evaluation of the bursa can be used to identify acute to subacute (3-5 days post-infection) bursal damage. There is a greater chance of detecting virus by molecular methods during the early stages of infection. Farms with past problems that may be associated with IBDV can be evaluated by weekly sampling of serum and bursa. In our experience, most IBDV bursal damage occurs between 3 and 4 weeks of age when maternal antibody has disappeared. A few farms have had bursal lesions present by 2 weeks of age. Samples of bursa and serum can be collected at 2, 3 and 4 weeks of age for evaluation by IBDxr serology and histopathology. Thymic lobes can also be evaluated for lymphoid depletion that may indicate other immunosuppressive agents (e.g., chicken anemia virus). At the time of sampling, please place ½ of the bursa in formalin and ½ of the bursa in a bag to freeze for possible future molecular testing. The cost for histopathology is $35.00/case.

If you have any questions regarding the tests described above, turnaround time, or sample submission, please contact Dr. Davor Ojkic at 519-824-4120, ext. 4524 or Dr. Brian Binnington at 519-824-4120, ext. 4550.

References

1. Jackwood DJ, Sommer SE. Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses. Avian Dis 1997; 41: 627-637.
2. Jackwood DJ, Sommer SE. Restriction fragment length polymorphisms in the VP2 gene of infectious bursal disease viruses from outside the United States. Avian Dis 1999; 43: 310-314.

SWINE

PRRS virus sequence analysis now available at the AHL

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The AHL continues to offer PCR identification of PRRS virus using the highly conserved nucleoprotein ORF7 gene region and restriction fragment length polymorphism (RFLP) typing of PRRS virus using the variable ORF5 envelope gene region. We now also offer sequence analysis of ORF5 gene products. **PCR sequence analysis is used to compare the sequence of various PRRS virus strains**. This allows the study of the epidemiology of PRRS virus within a herd or between herds. Both homogeneously similar and genetically diverse groups of PRRS virus isolates can simultaneously be present in the same swine herd. Viruses that are 98-99% homogeneous can be presumed to be strongly related (1).

Sequence analysis gives an estimate of sequence homology with other PRRS viruses by assessing percent identity to the closest PRRS virus ORF5 sequence match listed within GenBank. GenBank sequences are submitted from all over the world.

Sequences can also be compared in table format with other sequences from the same herd or from different herds, with vaccine viruses or with a selected subset of the 125 Ontario PRRS sequences previously determined at the University of Guelph over the last 4 years. Percent identity and divergence are compared between viruses (Table 1).

Table 1. Percent identity and divergence of selected PRRS viruses in comparison to PRRS virus vaccine strain ResPRRSV and Prime Pac. The RFLP ORF5 typing pattern is listed after the AHL case number.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1		99.7	90.9	88.7	87.2	88.2	97.0	89.2	86.4	87.2	96.2	88.6	87.4	86.9	97.3	**1**	ResPRRSV ORF5-252
2	0.3		91.2	89.2	87.7	88.2	97.3	89.6	86.9	87.2	96.2	89.1	87.6	86.9	97.7	**2**	VR-2332-252
3	8.9	8.5		88.9	89.1	89.1	90.0	90.0	87.9	88.1	88.4	88.6	88.9	88.6	90.4	**3**	Prime Pac ORF5-144
4	10.7	10.3	11.6		91.5	87.7	86.9	90.2	91.5	87.9	87.7	95.9	91.2	87.6	87.9	**4**	G98-38103-123
5	12.0	11.6	11.4	7.8		91.2	86.2	90.4	98.5	92.5	84.7	92.5	98.3	91.2	86.7	**5**	G98-01682-134
6	11.1	11.1	11.1	12.5	9.2		87.1	90.2	91.0	94.0	87.2	87.7	91.4	95.9	88.1	**6**	G98-05038-172
7	3.1	2.7	9.8	13.1	13.8	12.7		88.1	85.4	86.2	93.5	86.4	86.1	85.7	95.9	**7**	G98-00606-252
8	10.7	10.3	10.3	9.7	9.7	10.1	11.9		89.6	89.6	88.1	90.2	89.4	90.2	88.6	**8**	G99-1660-123
9	13.1	12.7	12.2	8.3	1.5	9.4	14.9	10.7		91.4	85.2	90.7	97.7	90.9	86.1	**9**	G99-60178-134
10	12.7	12.7	12.3	12.5	8.0	6.0	14.1	11.3	9.0		85.4	88.7	91.7	93.9	87.4	**10**	G99-02224-171
11	3.8	3.8	11.2	12.3	14.7	12.5	6.6	11.9	14.9	14.4		86.7	85.6	86.7	95.0	**11**	G99-00422-252
12	11.1	10.7	11.8	3.9	6.8	12.3	13.5	9.9	8.5	11.5	13.2		92.2	88.1	87.7	**12**	G00-20322A-123
13	11.8	11.8	11.4	8.5	1.7	9.1	14.0	10.8	2.2	8.8	14.5	7.2		91.0	86.6	**13**	G00-18697-134
14	12.5	12.5	11.4	12.7	9.1	4.3	14.1	10.1	9.5	5.9	13.1	12.1	9.5		87.2	**14**	G00-16299-172
15	2.5	2.2	9.3	11.5	13.1	11.5	4.1	11.5	13.7	12.7	5.0	12.4	12.8	12.5		**15**	G00-13056-252
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**		

(continued on page 33)

Sequence analysis results can also be presented as a phylogenetic tree, with the distances between the branches an indication of the degree of homology between virus strains (Figure 1). Closely related viruses will be on the same branch, or on a nearby branch. In most cases, viruses with similar RFLP typing patterns are on the same branch. However this may not always be the case, as for G99-1660-123, which is not grouped with other RFLP 123 types.

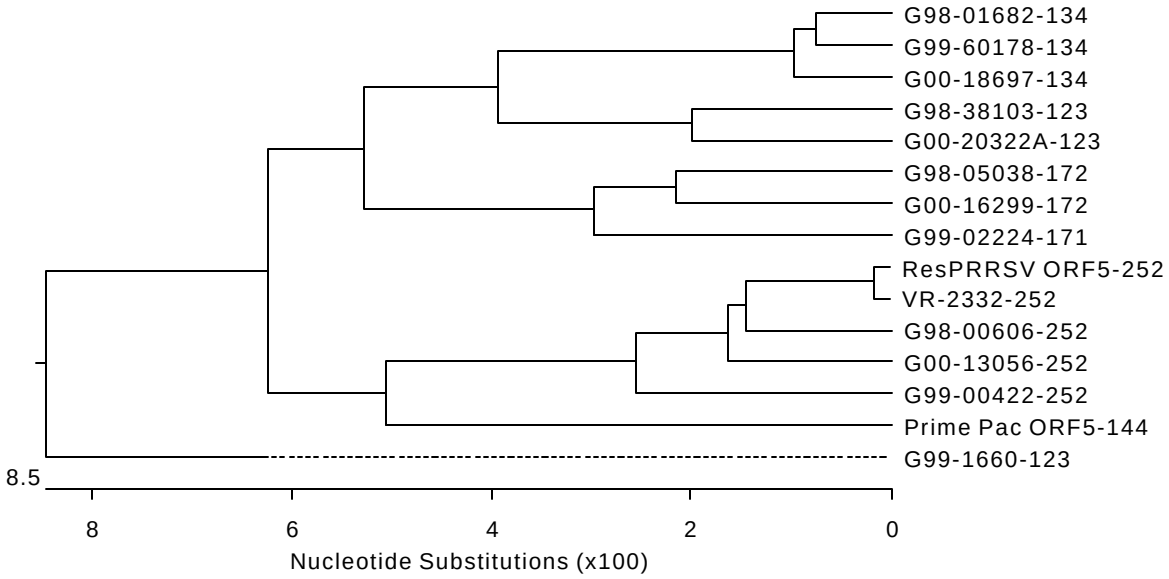

Figure 1. Phylogenetic tree of PRRSV ORF5 sequences selected from 125 cases previously sequenced at the University of Guelph. The RFLP ORF5 typing pattern is listed after the AHL case number.

Samples that are positive in the initial ORF7 PCR and in the subsequent ORF5 typing PCR are suitable for PCR sequencing and sequence analysis. The fee for PCR sequencing and sequence analysis is $200/test. For more information, contact Dr. Susy Carman (519) 824-4120, ext 4551.

Reference

1. Dee S, Torremorell M, Rossow K, et al. Identification of genetically diverse sequences (ORF5) of porcine reproductive and respiratory syndrome virus in a swine herd. Can J Vet Res 2002; 65: 254-260.

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HORSES

Equine abortions, 2001/2002

Beverly McEwen, Marie Archambault, Susy Carman, Murray Hazlett *bmcewen@lsd.uoguelph.ca*

The number of equine abortion submissions was at an all-time high in May 2001 to April 2002 (Table 1), but the 97 cases accounted for 1.5% of total equine submissions, which is unchanged from the last 2 years. The cases were submitted from over 60 owners, however, owner identification was not given in 29 cases, making diagnoses at the herd-level problematic. Eleven breeds were represented. Thoroughbred abortion submissions were over-represented at 30% compared to the overall AHL case submission rate of 23%, whereas Standardbred abortion submissions of 28% were lower than the overall AHL submission rate for Standardbreds of 32%.

Infectious abortion collectively comprised 32% of all abortion diagnoses, followed by non-infectious causes of abortion, primarily umbilical torsion (15%). Of infectious causes, **equine herpesvirus type 1(EHV-1) remains the most frequent single abortifacient pathogen identified**, accounting for 10% of all abortion diagnoses. Only two of the EHV-1 abortions were from the same premises. Except for the absence of cases with placental edema this year, equine abortion diagnoses have not changed substantially over the past 4 years.

Table 1. Equine abortion diagnoses, AHL fiscal years 1998/1999 - 2001/2002

Fiscal year	**98/99**	**99/00**	**00/01**	**01/02**
# of abortion cases submitted	64	89	89	97
Frequency of abortion of total AHL equine submissions	1.32%	1.69%	1.50%	1.54%
Pathology diagnoses (number,%)*				
EHV-1	**9**	**8**	**13**	**10**
Proportion Equine abortions EHV-1	14%	9%	15%	10%
Non-viral infectious abortion , combined	**17**	**25**	**16**	**21**
Proportion non-viral infectious abortion	27%	28%	18%	22%
Streptococcus zooepidemicus	1	5	1	4
Staphylococcus aureus	0	3	0	0
Streptococcus equisimilis	2	0	0	1
Ehrlichia risticii	0	0	0	0
Klebsiella spp	0	0	1	0
Leptospira spp	3	0	0	1
Nocardia spp	0	0	1	0
Placentitis	8	7	6	11
Miscellaneous bacteria/fungi	2	8	0	0
Mycotic	1	0	1	2
Lesions compatible with bacterial	0	2	6	2
Non-infectious causes of abortion, combined	**15**	**21**	**24**	**26**
Proportion, non-infectious causes of abortion	23%	24%	27%	27%
Umbilical torsion	8	12	8	15
Placental edema	4	3	8	0
Placental mineralization	1	0	0	4
Placental adenomatous/cystic hyperplasia	0	1	0	0
Fetal goiter	0	0	1	1
Dystocia/stillbirth	2	5	7	6
Congenital anomalies	0	0	0	0
Idiopathic	**24**	**38**	**36**	**42**
Proportion idiopathic	38%	43%	40%	43%

* Number of diagnoses may add to more than the number of cases submitted because more than one diagnosis was made in some cases.

COMPANION ANIMALS

Feline infectious peritonitis: Testing options, interpretation, and usefulness

Josepha DeLay, Susy Carman, Stephen Kruth *jdelay@lsd.uoguelph.ca*

The diagnosis of feline infectious peritonitis (FIP) remains difficult despite new insights into the causes of this devastating disease and the development of more sophisticated testing procedures to detect the virus responsible for this condition. FIP is caused by a virulent feline coronavirus (FCoV), feline infectious peritonitis virus (FIPV), which arises by mutation of relatively avirulent feline enteric coronavirus (FECV), a common virus transmitted mainly by the fecal-oral route and resulting in inapparent infection or mild diarrhea. The mutation promoting virulence is thought to arise within cats already infected with FECV, suggesting that FIPV itself is not horizontally transmitted between cats. The virulent new virus is better able to replicate in blood monocytes and in tissue macrophages. An individual cat's age at time of infection, immune response to the virus, and possibly other inherited factors also influence whether or not a cat harboring FIPV will develop the disease. **It is important for practitioners to differentiate between FIP (a syndrome of immune complex vasculitis and pyogranulomatous inflammation), FECV infection, and FIPV infection, and to understand that serological testing or RT-PCR cannot differentiate between the two viruses.**

RNA viruses such as coronaviruses are notorious for their poor replication fidelity, resulting in frequent mutations. Several different mutations can give rise to the virulent form of virus associated with FIP and the various mutations tend to have specific cattery or geographic distribution. However, to date, no consistent mutations have been correlated with the development of FIP. Cats infected with FIPV cannot be differentiated serologically from those infected with FECV, as antigenic similarities in the two viruses produce cross-reacting antibodies. Similarly, the sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) used to identify FCoV RNA in tissues, blood, body fluids, and feces cannot differentiate between virulent FIPV and avirulent FECV. RT-PCR can be used to identify cats that are chronic shedders of FECV and who therefore increase the risk of FECV infection and eventual mutation to FIPV among other cats in a cattery or household.

> Antemortem diagnosis continues to rely on combined information from the history, clinical signs, and laboratory data

Results of RT-PCR for FCoV in healthy cats must be interpreted with even greater caution. In one study, 26% of healthy cats were RT-PCR positive for FCoV in blood or plasma. Results of RT-PCR on single samples can be misleading, and chronic FECV-shedding cats can be identified only after demonstration of continuous viral presence in multiple fecal samples over a period longer than 9 months.

Useful tests in FIP diagnosis:

Antemortem diagnosis continues to rely on **combined information from the history, clinical signs, and laboratory data:**

1. ***Clinical pathology*.**
   a. Exudates with low cellularity and high-protein content (>35 g /L) may be demonstrated in body cavities.
   b. Serum hyperproteinemia may also be detected.

2. ***Serology*.** Antibody titers are meaningless for diagnosis and prognosis of FIP. A positive ELISA or immunofluorescence assay (IFA) for antibody to FCoV indicates current or recent exposure to these viruses, but **cannot differentiate between FECV and FIPV exposure**. In addition, a negative serological result does not preclude a diagnosis of FIP.

3. ***RT-PCR ("DNA test").*** This sensitive test detects feline coronavirus RNA in effusions, blood / plasma, or feces. **Positive RT-PCR results are indicative only of FCoV infection and may represent either FIPV or FECV infection.** Positive identification of FCoV genetic material in body cavity effusions can support a diagnosis of FIP, but only in conjunction with other test results indicative of this disease. A single positive RT-PCR result in a healthy cat can be misleading and should not be used as a justification for euthanasia, as the great majority of these animals will not develop FIP.

4. ***Histopathology* biopsies and necropsy samples remains the only definitive test for FIP**. Pyogranulomatous vasculitis and perivasculitis in various tissues are consistent with this diagnosis. *Fluorescent antibody* and *immunohistochemistry antigen detection tests* on these samples are helpful adjuncts to histopathology.

(continued on page 36)

Feline infectious peritonitis *(continued)*

References:
1. Addie DD, Jarrett O. Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats. Vet Rec 2001; 148: 649-653.
2. Kennedy MA, Brenneman K, Millsaps RK, Black J, Potgieter LND. Correlation of genomic detection of feline coronavirus with various diagnostic assays for feline infectious peritonitis. J Vet Diagn Invest 1998; 10: 93-97.
3. Vennema H, Poland A, Foley J, Pedersen NC. Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology 1998; 243: 150-157.

An interesting review of feline infectious peritonitis can be found in Veterinary Sciences Tomorrow, Issue 1, January 2001 at www.vetscite.org

Nutritional osteodystrophy revisited

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We have received numerous email and telephone comments following publication in the June 2002 AHL Newsletter of a case report of nutritional osteodystrophy in puppies fed a "biologically appropriate raw food" or "bones and raw food" ("BARF") diet. **Nutritional analysis underway at the time of initial publication has identified inappropriate calcium to phosphorus ratios, ranging from 1:5.7 to 1:10, in samples of the diet fed**; the ideal dietary Ca:P ratio for most species is 1:1. In addition, **the diet contained deficient levels of calcium**. The results of analyses for these two macrominerals confirm nutritionally induced secondary hyperparathyroidism as the cause of severe bone abnormalities in these puppies.

Many canine raw-food diets are available on the Internet and through other sources, but evidence of the beneficial effects of these diets remains anecdotal. In addition to diseases and disorders induced by nutritional imbalance, bacterial infection of dogs, owners, and the environment with such organisms as *Salmonella* spp. and *Escherichia coli* O157:H7 is an additional and serious concern in feeding raw meat products.

Veterinarians and dog owners may find the following publications useful in learning more about the issues involved in canine raw food diets:

Billinghurst, I. The BARF Diet. Alexandria, Australia: SOS Printing Pty. Ltd., 2001.

Freeman L, Michel KE. Evaluation of raw food diets for dogs. J Am Vet Med Assoc 2001; 218: 705-709.

Joffe DJ, Schlesinger DP. Preliminary assessment of the risk of *Salmonella* infection in dogs fed raw chicken diets. Can Vet J 2002; 43: 441-442.

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