LABORATORY SERVICES Newsletter
ANIMAL HEALTH LABORATORY GUELPH KEMPTVILLE RIDGETOWN

Volume 2, Number 2

In this issue:

What's happening at the AHL?

- **Dr. Brian Binnington** joins the AHL-Guelph on June 15 as a pathologist in the avian/fur-bearers section. Brian worked as a pathologist, and latterly as lab head, in the Brighton VLSB lab prior to its closure in 1995, and brings a wealth of experience to the position. Our thanks to **Dr. Bob Hampson** for filling in for the last year.
- **Dr. Rob Bildfell**, mammalian pathologist, has resigned to take up a position at Oregon State University.
- **Mr. Nick Schrier** has joined the AHL as toxicology scientist. Nick worked in the U of G Department of Chemistry and Biochemistry for 11 years, and was recently awarded an MSc in chemistry.
- We continue to accept "incoming collect" courier shipments *from within Ontario* at **no charge to you** if you quote the U of G account number from page 4 of the 1998 AHL Fee Schedule (e.g. Purolator - 6772212).
- Our **updated fee schedule** came into effect May 1/98. Please note one change: **Virology, mammalian BVDV antigen ELISA is $10/animal, not $10/case**
- We are now using the IDEXX kit to provide **bovine *Neospora* antibody ELISA** results. The test fee is **$6.00 per test.**
- The AHL and the OVC currently have limited availability of staff for **parasitology consultations** with practitioners as a result of recent events. **Dr. Phil Lautenslager** retired from VLSB prior to the enhanced partnership with the UofG, and **Dr. Owen Slocombe** recently retired from the OVC. **Drs. John Barta** and **Ramon Carreno** are providing parasite identification backup to technicians **Ron Ford** and **Julie Cobean**, but cannot provide consultations. We are referring food animal parasite control questions to Health Management, OMAF, Fergus. The OVC Small Animal clinicians are only able to provide consultations on in-clinic cases. Answers to commonly asked heartworm questions are available in Dr. Slocombe's annual heartworm survey results, mailed with the latest CVO Update. AHL

Feedback for the AHL?

Please feel free to call, fax, or E-mail us at any of our labs.

SMALL ANIMALS

Diagnostic testing for canine hypothyroidism - new tests

Dr. Brent Hoff

1) Serum free T4 by dialysis (FT4D) concentration

Free T4 is the fraction of the thyroid hormone thyroxine (T4) that is not bound to protein. The free fraction of T4 is less influenced than the bound fraction by factors such as non-thyroidal illness (NTI) or drug therapy that may falsely lower T4 concentrations. An equilibrium dialysis FT4 assay is now available, and is a more sensitive and specific diagnostic test for hypothyroidism.

Measurement of FT4D can also be used to monitor thyroid supplementation, but offers no advantage over T4 levels, except in cases of thyroiditis in which serum T4 levels are falsely elevated by the presence of T4 autoantibodies.

2) Canine thyroid-stimulating hormone (cTSH)

A validated assay for canine TSH is now available. The diagnostic accuracy of a single TSH measurement is approximately 90 percent. When both endogenous TSH and FT4D measurements concur, the diagnostic accuracy approaches 100 percent. **Therefore, dogs with advanced thyroiditis or idiopathic hypothyroidism should have low FT4D concentrations and elevated cTSH levels.**

Based on a study from The Animal Medical Center in New York, false-negative results (normal cTSH), occur in about 25 percent of hypothyroid dogs. False-positive results (elevated cTSH), occur in about 10 percent of dogs with NTI and occasionally in normal dogs. The reason for this in hypothyroid, sick euthyroid and healthy dogs remains unclear. Measurement of cTSH concentrations appears to be most useful when used in conjunction with FT4D or TT4 levels to diagnose primary hypothyroidism.

3) Thyroglobulin autoantibodies (TgAA)

can be detected in about 50% of hypothyroid dogs. These autoantibodies are found in most cases of lymphocytic thyroiditis; the occurrence of antithyroid hormone antibodies (T3aa, T4aa) is less common. With few exceptions, dogs positive for T3aa or T4aa are positive for TgAA, while very few dogs that are positive for TgAA have any detectable T3aa or T4aa.

Presently, **many workers feel that hypothyroidism is over-diagnosed**. Other conditions may mimic the clinical signs of hypothyroidism, and false-positive test results (low T4) are common in euthyroid patients. These workers feel that FT4D and cTSH should be used to assess thyroid status, and the results of a positive TgAA test alone, without other evidence of hypothyroidism, should not be used to advise clients with regard to breeding.

Making the diagnosis: Recommendations

If the goal of testing is to diagnose hypothyroidism without regard to possible etiology, a combination of FT4D and cTSH would seem reasonable.

If the goal of testing is to determine a more complete picture of thyroid function (e.g. for possible genetic counseling, or entry into OFA registry), then a thyroid profile including TgAA, cTSH and FT4D is recommended. AHL

Small animal serology
Dr. Susy Carman

The routine yearly vaccination of small animals has become controversial. For some of your patients, you may wish to evaluate their antibody titers to viral agents prior to revaccination. To assist you, the Animal Health Laboratory offers virus neutralization (VN) and hemagglutination-inhibition (HI) tests for the assessment of antibody in serum to the following canine and feline viruses:

Canine
- Canine adenovirus-1/2 (laryngotracheitis/hepatitis) - VN
- Canine coronavirus - VN
- Canine distemper virus- VN
- Canine herpesvirus - VN
- Canine parvovirus-2 - HI

Feline
- Feline calicivirus - VN
- Feline herpesvirus - VN
- Feline parvovirus (panleukopenia) - HI

Please send 0.5 ml of serum for each test. VN tests are performed using cell culture and require a five-day incubation period. These tests are set up once a week. Charges are **$10 per test**. For more information please call **Dr. S. Carman** 519-824-4120 ext 4551. AHL

Urolith update
Drs. Grant Maxie and Arlene Yee

Analysis of companion animal uroliths is proving to be a very popular service! The Canadian Veterinary Urolith Center, officially launched at the OVMA convention in January, provides a collaborative service by the Laboratory Services Division and Veterinary Medical Diets. We have analyzed over 1000 calculi from across Canada. About half of the calculi have been submitted from Ontario, but all provinces are represented.

Based on the first 867 calculi analyzed, the most common uroliths in **dogs** were magnesium ammonium phosphate hexahydrate (n = 401), calcium oxalate dihydrate (76), calcium oxalate monohydrate (44), and calcium phosphate, apatite form (31). The prevalence was similar in cats - magnesium ammonium phosphate hexahydrate (n = 210), calcium oxalate monohydrate (33), calcium oxalate dihydrate (29).

CATTLE

Warm weather milk
Dr. Dan Stevenson

Summer is here, with its heat and with spoiled sample concerns. Contaminated, unreadable milk samples are a frustration for all involved. The number of contaminated milk samples increases considerably during the warm summer months. Proper collection is always important but **cooling immediately after collection** and keeping the samples chilled until they get to the lab are key. Frozen samples are not suitable for CMT testing but are fine for regular culture on individual and acute samples.

Styrofoam coolers with ice packs are best for shipping samples. An alternative is to place ice packs

directly against the milk box(es) and wrap them together in newspaper within a cardboard box for shipping. In-transit shipping delays are beyond our control. Whenever possible, shipping early in the week may help reduce the complications of short-term in-transit delays. Remember to include our UofG courier account number (on page 4 of May/98 AHL Fee Schedule booklet) on the courier waybill to ship "incoming collect". AHL

SWINE

Update on PRRSV RT-PCR
Dr. Hazel Alexander

It has been three months since we began offering the reverse transcription-polymerase chain reaction (RT-PCR) for the diagnosis of porcine reproductive and respiratory syndrome virus. We have tested 151 tissue samples, of which there were 54 positive samples (35%), and 91 serum samples, of which 14 were positive (15%). On a per case basis, 38% of the cases were positive. The agreement between PCR and FA was evaluated by determining the Kappa value. The Kappa value was 0.74, indicating a good level of agreement (Kappa values of 0.8-1.0 indicate excellent agreement).

Positive samples were genotyped using the method described by Dr. Wesley (USDA, Ames, Iowa) from the ORF 5 area of the genome. The pattern corresponding to the Resp/PRRS vaccine or its parental strain, **VR2332**, was found in 18 of the 36 samples tested (50%). There were 11 different patterns seen from the remaining samples. Of these, 11% produced patterns which were close to, but not identical to, the vaccine strain. These patterns will be reported as **intermediate strains**. Please see the paper in the Proceedings of the American Association of Swine Practitioners, 1998 by Dr. Wesley regarding these intermediate strains. The pattern of the Prime-Pac vaccine strain using this method is now known. Unfortunately, this pattern is not unique. **It will be very helpful to us if you include the trade name of any PRRSV vaccines being used in the herd in the history on the submission sheet.**

Work is currently underway to evaluate a primer set for PCR detection of **porcine circovirus**. Samples which are histologically suggestive of the disease will be tested, as will samples which are not suggestive of the disease. We will keep you informed as to when this test will be available. AHL

Actinobacillus pleuropneumoniae serotyping
Dr. Gaylan Josephson

A 16-month review of VLS/AHL files (Jan 1/97-May 1/98) identified a total of 74 submissions from which *Actinobacillus pleuropneumoniae* had been isolated. These submissions originated from 53 premises. Serotyping had been attempted on 51 of the isolates (from 37 premises) (Table 1). Multiple submissions from one producer over a 12-month period identified the presence of serotype 5 only; submissions from another producer over 6 months identified the continued presence of serotype 1.

Table 1. APP serotypes identified in this study, compared to 1993 Canadian results

Serotype	Results from this study*	1993 Canadian results**
1	16 (38%)	30%
5	13 (31%)	44%
7	12 (28%)	12%
Untypable	1 (2%)	

* Isolates from 3 different premises were identified as belonging to serotypes 1 and 5, and from 2 other premises as being serotypes 1 and 7

** Gottschalk M, Bilodeau R. Detecting carrier animals in herds chronically infected by *Actinobacillus pleuropneumoniae:* the detection of antibodies and the detection of the bacteria. 1995 Allen D. Leman Swine Conference

Total mortality, either in numbers or percentages, could not be determined without an extensive follow-up investigation. Histories were often inadequate, but when mentioned, death losses ranged from less than 1% (3 of 1500 animals) to 11.5% (23 of 200) at time of submission.

Antibodies against different serotypes of APP are commonly found in a high proportion of swine herds. In addition, antibodies against more than one serotype can be identified within the same herd, and indeed, within the same animal.

These results suggest that serological profiling for the detection of antibodies against APP should be conducted against at least serotypes 1, 5 and 7. AHL

K88 strains of *E. coli*

Drs. Gaylan Josephson and Nonie Smart

Pigs with enteric colibacillosis are often found dead or moribund with or without evidence of diarrhea. Pooling of fluid, occasionally blood-stained, is noted on necropsy examination, along with venous infarction of the stomach. Histologically, gram-negative bacteria consistent with *E. coli* are seen to adhere to the epithelium of the small intestine. Microthrombi are also occasionally apparent in other organs.

Experimentally, gnotobiotic pigs with enteric colibacillosis and hemorrhagic enteritis are usually endotoxemic and bacteremic (this latter may be a terminal event). Peracute colibacillosis with endotoxic shock is most often seen in pigs infected with K88 strains of enterotoxigenic *E. coli* (ETEC strains), which are hemolytic on blood agar. F18 (edema disease) strains may also produce a somewhat similar clinical picture, but isolates from the AHL microbiology laboratory are almost always hemolytic, in pure culture, and identified as K88+ ETEC, indicating that the edema disease producing strains are not likely to be part of the microbiological picture (1). In addition, edema disease (gut edema), in its classical form, appears to be extemely rare in Ontario, having been identified in only one herd in the past several years (this herd is an experimental herd, not a commercial enterprise).

The Animal Health Laboratory has identified an almost 3-fold increase in the isolation of K88+ organisms from piglets over the last year (see Table 1). While K88+ ETEC organisms have traditionally been thought to be associated with post-weaning diarrheas, the organism has been identified in suckling piglets as young as 2-3 days of age. The majority of the isolates are from clinical cases of diarrhea in post-weaning pigs (Figure 1), however, with early weaning practices (under 3 weeks of age) often mentioned in the accompanying history.

Table 1. K88 *E. coli* isolations from piglets as identified in AHL records, from Oct 1/96 - May 20/97, and from Oct 1/97 - May 20/98.

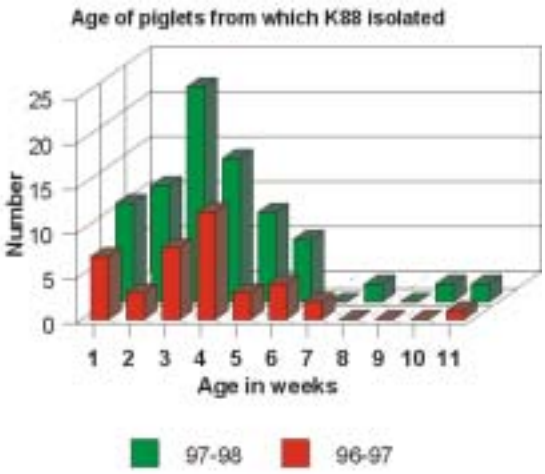
	1996-97	1997-98
Total # of swine submissions	978	1349
Total # of enteric submissions	233	299

Total # of K88 isolates *	48	125
Average age of pig with K88 isolate	3.67 wk	3.85 wk
Age range	2 da - 11 wk	3 da -20 wk

* - Although sometimes in combination with other ETEC strains, the majority of recent isolates are pure cultures of hemolytic *E. coli* which are often autoagglutinating.

In the laboratory, we note that most K88+ isolates are observed as pure cultures of strongly hemolytic *E. coli* which often autoagglutinate in saline. For this reason, they cannot be further screened by antisera for reactions to other ETEC types. This type of culture finding appears to be strongly associated with clinical disease, as we have not to date observed this from submissions where "normal" animals are being screened by culture for possible K88+ *E. coli* carriage. When source piglets are being cultured in an attempt to predict which batches of piglets might be affected, we most often isolate mixed strains of *E. coli.*

Figure 1. Ages of piglets from which K88 *E. coli* were isolated.



The reason for this marked increase is not known, although one can speculate. It is possible that the increase in K88+ *E. coli* diarrhea in post-weaning pigs is due, at least in part, to the increased stress placed on pigs by early weaning procedures. The dietary changes that are an important factor in the adoption of segregated early weaning technology may also play an important role in pathogenesis. This time frame also coincides with the decrease in maternal antibody in the piglet, the loss of lactogenic antibodies following cessation of suckling, and a possible buildup of ETEC organisms in the environment. AHL

1. Moxley RA. Hemolytic strains of enterotoxigenic E. coli. University of Kentucky Herd Health Memo, No 8, 1997- 98.

Animal Health Laboratory Accreditations:

American Association of Veterinary Laboratory Diagnosticians (AAVLD) (lab system)
Thyroid Registry of the Orthopedic Foundation for Animals Inc. (OFA) (thyroid function)
Canadian Food Inspection Agency (CFIA) (EBL, EIA, bovine brucellosis)
Canadian Association of Environmental Analytical Laboratories (CAEAL) (metals)

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If you would like to be added to, or removed from the AHL Newsletter mailing list, please fax your request to Ms. Helen Oliver at 519-821-8072 or E-mail to holiver@lsd.uoguelph.ca

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