UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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A new year, and a new look

Grant Maxie

In order to make the AHL Newsletter as attractive and reader-friendly as possible, we've re-designed our layout to include:

- More space on the front page for important announcements.
- Reduced table of contents to save space
- Call-outs within articles to highlight important summaries or conclusions
- Boxed items for important reminders

Contents will remain similar to those of the past 5 years. Information on how to better use the lab will remain up front; scientific lab-based reports will follow.

> The AHL Newsletter is published to serve you - our client or partner!

My thanks to Laura Clark for producing a new template for the Newsletter and for getting me started on MS Publisher.

We welcome your feedback. Please contact us as indicated in the Newsletter masthead with suggestions for improvements, or ideas for articles. The AHL Newsletter is published to serve you - our client or partner!

Taking the pulse of client service

Grant Maxie

Through the Laboratory Services Quality Assurance Unit, the AHL measures service delivery via an **annual questionnaire** sent to ~50 key clients. Over 80% of respondents were either delighted or pleased with our service, 100% would use our service again, 96% would recommend our service to others, and 88% felt that they received value for fees charged.

We also send out a '**Service Check**' form with every statement. Our overall client service rating by this vehicle is 80-90%. As we continue to bill producer clients at the request of veterinarians, producers also receive Service Checks, and we receive occasional notes of frustration - "by the time I received results, the calf was dead", "you need a PhD to interpret the report", "please give treatment advice", "turnaround time was slow". As we do for client veterinarians, we fax results as they are produced to producers with fax machines, but 'mail clients' receive only a final printed report. We also send out with final reports the handout "Information for Owners", which clearly states turnaround times, and we state on every AHL report "For interpretation of results, please contact your veterinarian". Veterinarians are our primary clients.

We sincerely appreciate all efforts extended by veterinarians to report and interpret lab findings to their clients as promptly as possible.

We're on the Web! http://ahl.uoguelph.ca

Specimen submission - to freeze or not to freeze?

Peter Lusis *plusis@lsd.uoguelph.ca*

We often receive specimens that are unsuitable for testing because of improper preparation and/or shipping. This prevents accurate diagnosis and can lead to false positive or negative results in some cases. With the exceptions noted below, **specimens should be chilled or frozen as soon as possible after collection, and shipped on ice packs to arrive at the laboratory within 24 hours or less if at all possible**.

- For small animal **whole carcass** submissions, chilling is preferable to freezing (which is especially damaging to brain and other nervous tissue). Carcasses that are rapidly chilled after death can be kept for 2-3 days at refrigerator temperatures with minimal tissue damage.
- In cold weather, tissues for **histopathology** should be fixed in formalin prior to shipment to prevent freezing artifact.
- **Bacteriology and mycology** specimens can be frozen, except for isolation of **anaerobes**, which should not be frozen.
- **Milk** for bacteriology only should be chilled or frozen as soon as possible after collection, but must not be frozen if **somatic cell counts or mycoplasma isolation** are required.

Help us to help you in providing an accurate and meaningful diagnosis!

- **Parasitology** samples can be frozen unless *Giardia*, *Trichomonas* or other flagellates are suspected, in which case samples should be kept at body temperature and examined as soon as possible (within hours) after collection.
- **Virology** samples should be frozen as soon as possible after collection, **except for EDTA blood for virus isolation, and tissue for chlamydia isolation**, which should be chilled and submitted as soon as possible after collection. Separated serum for **serology** can be submitted chilled or frozen.
- **Mycoplasma/ureaplasma** samples should be chilled and submitted as soon as possible after collection; they should be frozen only if a delay in shipment is anticipated.
- **Toxicology and biochemistry** samples can be frozen, **except when EDTA blood is required** (for selenium, etc.).
- **Hematology** samples should be fresh or chilled, and blood smears made as soon as possible after collection.

Help us to help you in providing an accurate and meaningful diagnosis!

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Assessment of trace minerals in practice

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To assess the mineral status of animals, you begin with the usual diagnostic procedures used to diagnose any condition in veterinary medicine. Important clues may come from recording an accurate clinical history, observing clinical signs, results from clinical pathology screens, post mortem examinations, as well as obtaining the proper samples for trace mineral testing. Interpretation of trace mineral levels in biological samples must be done in light of the animal, history, and sample type.

Clinically, a trace mineral deficiency or excess may appear as a primary disease, or may be subtle, manifested as an increased incidence of other diseases. Examples of primary problems include inherited copper storage disease in Bedlington terriers and West Highland White terriers, and nutritional myopathy (white muscle disease) with vitamin E and/or selenium deficiency in calves, lambs, pigs, and foals. Ruminants are very sensitive to the copper/molybdenum balance in the ration and high sulfates will enhance that interaction. Sheep are very sensitive to both copper excess and deficiency. Pet birds are sensitive to zinc and lead excess as well as to iron storage disease. Young animals of all species are sensitive to mineral imbalances, which can result in problems such as "swayback" in lambs.

Signs may be very specific, such as ataxia with demyelination due to copper deficiency in sheep. The problem may be very subtle, as in cattle with copper deficiency/ molybdenum excess, with ill-thrift, poor hair coat, and diarrhea. Animals may appear clinically normal during the accumulation phase. The metals may be suddenly released from liver storage to the kidney. Some animals may have impaired immune systems, and increased incidence of infectious disease or "failure of vaccination".

The assessment of mineral status often relies on accurate mineral analysis of appropriate animal and feed samples, and knowledgeable interpretation of the results. Sampling of serum may indicate a problem with copper, zinc, iron and trace salt status of animals. Samples for zinc analysis must not contact rubber stoppers, therefore trace-mineral tubes (royal blue top) are required. Whole blood samples are useful for selenium and manganese. Liver is definitely the best sample for assessing the copper status in animals - a small Tru-Cut sample will suffice as a biopsy.

> The AHL offers a trace mineral and a toxic metal panel for blood/ serum, liver and feed samples.

The AHL offers a trace mineral and a toxic metal panel for blood/serum, liver and feed samples. Samples are analyzed using atomic absorption spectroscopy (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), or fluorescence methods. Interpretation of results is done using all other findings, including history and pathology results. Please call Dr. Hoff at 519-824-4120, ext. 4527, if you need assistance with one of these cases.

Two new tests for *Escherichia coli* genotyping are available at the AHL

Hugh Cai, Marie Archambault *hcai@lsd.uoguelph.ca*

Verotoxigenic (or Shiga toxigenic) *Escherichia coli* (VTEC or STEC) produce Shiga toxin type 1 (Stx1) and/or Shiga toxin type 2 (Stx2), which are encoded by stx_1 and stx_2 respectively. VTEC strains that produce accessory virulence factors such as intimin (encoded by *eaeA*) and enterohemolysin (encoded by *hlyA*) are likely to have a higher degree of virulence for humans. Those producing Stx2 may cause more serious complications than those producing only Stx1. *E. coli* serotypes O111 and O157 belong to the VTEC (or STEC) family, which produce Shiga toxin, and appear to be of greater virulence for humans. Although unusual, VTEC has been associated with diarrhea and colitis in calves (1).

- We have adapted a published multiplex PCR method to detect the presence of stx_1, stx_2 (including variants of stx_2), *eaeA* and *hlyA* (2), so as to identify VTEC. Please indicate "**VTEC PCR genotyping"** when requesting this test.
- We have also implemented a multiplex PCR for detection of *E. coli* O111 and O157, which amplifies specifically the O-antigen gene, *rfb*. Please indicate "**O157/ O111 PCR typing"** when requesting this test.
- **The fee for each of the above tests is $25/sample for food animals and $50/sample for non-food animals.**

Please forward both your request and your sample to the bacteriology laboratory, as genotyping is performed on *E. coli* cultures. To enquire about the test, please contact the AHL at 519-824-4120 for Drs. Marie Archambault (ext 4536) and Hugh Cai (ext 4316).

References:

1. AHL Newsletter 2001; 5 (2): 25-26.
2. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, eaeA, enterohemorrhagic *E. coli* hlyA, rfb_{0111}, and rfb_{0157}. J Clin Microbiol 1998; 36: 598-602.

AHL Lab Reports

CATTLE

Bovine abortion diagnostics: Maximum benefit for your client's dollar

Josepha DeLay *jdelay@lsd.uoguelph.ca*

The quality and selection of samples you submit to us are the single most important factors in reaching a diagnosis in abortion cases. Abortion work-ups can be frustrating for clients, veterinarians, and diagnosticians when no specific etiologic diagnosis is reached. A consistent and thorough approach to abortion cases can maximize your information yield, and provide specific information to your client regarding potential management strategies to reduce abortion losses. The main goal of an abortion work-up is to determine if a potentially controllable infectious (or other) disease is responsible for fetal losses. In this context, negative results from a thorough battery of diagnostic tests are as useful as positive tests, as these can rule out involvement of many agents.

Submission of a complete fetus and placenta for necropsy examination can dramatically improve the chances of reaching a specific diagnosis in abortion due to infectious causes. At the Animal Health Laboratory, 235 bovine abortion cases were submitted in the year 2000; of these, 54% were submitted as necropsy cases, and samples were collected by the referring veterinarian in the remaining 46% of cases. **A specific etiologic diagnosis was reached in 44% of necropsy cases, but in only 27% of mail-in submissions.** Organ sampling and test selection varied greatly among the mail-in cases, indicating that consistency and completeness of sampling was likely the most significant contributing factor to this variation between the two groups of submissions.

Submitted intact fetuses with placentas are routinely sampled for histology, bacteriology, mycoplasmology, and virology, in addition to routine necropsy examination. A strict protocol is followed for sample submission to each specialty lab area in order to maximize the chances of identifying organisms that may be present, with the tissues sampled reflecting expected distribution of infectious agents commonly involved in bovine abortions. For example, samples for bacteriology and mycoplasmology include placenta, lung, and stomach content because placental infection often results in aspiration and swallowing of contaminated amniotic fluid, with dissemination of the organism to the lung and stomach. Samples for histology and virus isolation are selected with similar pathogenetic mechanisms in mind. Frequently, we identify histologic lesions suspicious for an infectious cause of abortion, but we cannot be more definitive because appropriate tissues for organism isolation were not submitted.

What can YOU do to improve your clients' information return on their diagnostic dollar?

- **Consider submitting the entire fetus and placenta** to the AHL for necropsy and additional testing.
- If sampling on the farm for mail-in submission, **be thorough and consistent in examination of fetus and placenta, in sampling, and in test selection**. A list of tissue samples and tests can be found on pages 11 and 12 of the AHL User's Guide.
- **Place EACH tissue for EACH test in a separate Whirl-Pak bag.** Mixing tissues and dividing samples after submission increase the chances of contamination, thus decreasing the likelihood of reaching a useful etiologic diagnosis.
- **Include brain with fixed tissues for histology.** Brain lesions are evident in approximately 95% of fetal *Neospora* infections, making it well worth the effort to expose and remove the brain. Skeletal muscle, heart, liver, and placenta also frequently have diagnostic lesions in *Neospora* cases, and should be included in the submission.
- **Avoid using swabs**, which can be easily contaminated. Submission of tissue samples is most satisfactory.
- If you choose not to request all tests, freeze and save the samples at your clinic for possible future submission. For the cost of a few Whirl-Paks, you may be rewarded with a diagnostic answer at a later date!
- **Collect serum** from the dam, for submission or for storage if needed in the future.

Jejunal hemorrhage syndrome in a Jersey cow

Margaret Stalker, George Wood

An adult Jersey cow was submitted for necropsy after a brief course of clinical disease (sudden onset of recumbency, subnormal temperature, bloat). The cow had temporarily responded to intravenous calcium and fluid replacement, but died 30 hours after onset of clinical signs.

The significant finding at necropsy was marked distension of a 2 m section of distal duodenum/proximal jejunum by a mass of clotted blood, originating from a 10 cm mucosal hematoma that obstructed the gut lumen. Histology revealed coagulation necrosis of small intestinal villi at the site of the hematoma, with infiltrating neutrophils, and invasion of large bacilli into the submucosal connective tissue. Bacterial culture revealed large numbers of *Clostridium perfringens* genotype A in the small intestinal content.

This cow represents the first case of so-called jejunal hemorrhage syndrome (JHS) (1) diagnosed at the AHL. This condition has recently been diagnosed with increasing frequency from several US states, including New York, Pennsylvania, Iowa, Minnesota, Wisconsin, Colorado, and Washington. The syndrome typically affects high-producing mature dairy cows from high-producing herds, and has a high case fatality rate. Cows may be found dead, others are recumbent and afebrile or cold for a short time before death. Other animals may have a longer clinical course with a sudden decrease in milk production, colic or ileus, and yellow diarrhea that turns black within 12 hours, or contains frank blood clots. Fevers have not been reported. Usually, cows are dead within 24 hours. At necropsy, these animals have distinct sections of jejunum distended by a large amount of frank hemorrhage with immediate clotting. *Clostridium perfringens* type A has been suspected to play a role in the development of this lesion, but the definitive etiology is not yet known. The epidemiology of this disease continues to be an important ongoing area of research in new and emerging diseases of dairy cattle (2). The website of the WADDL (listed below) includes a diagnostic protocol and questionnaire for veterinarians who may encounter this syndrome in the field.

This cow represents the first case of so-called jejunal hemorrhage syndrome (JHS) diagnosed at the AHL.

References

1. "Jejunal hemorrhage syndrome": A new, emerging disease of dairy cows? Washington Animal Disease Diagnostic Laboratory, 2000. http://www.vetmed.wsu.edu/depts_waddl/dx/jejunal_hemorrhage_syndrome.html
2. The Bovine Practitioner 35(2), 2001, pp. 97-103, 104-116.

Doxycycline cardiotoxicity in veal calves

Beverly McEwen, Peter Lusis, Marie Archambault, Tony van Dreumel *bmcewen@lsd.uoguelph.ca*

Sudden death of up to 10% of cohorts in a veal operation occurred over several months. The consistent and significant finding in the 7 calves necropsied was acute ventricular myocardial degeneration, mineralization and necrosis. All dead calves had been treated for enzootic pneumonia metaphylactically* for 3-4 days with doxycycline, a semi-synthetic derivative of tetracycline. Due to mixing errors, the calves had received 6-8 times the recommended dose.

Doxycycline is a broad-spectrum, bacteriostatic antibiotic that is absorbed well orally, even in the presence of calcium (milk). Doxycycline-induced myocardial degeneration, necrosis, and mineralization resulting in sudden death of veal calves have been reported in the Netherlands (1). The veal calves submitted to the AHL had therefore received a toxic dose of a reportedly cardiotoxic drug. **This is the first report that we are aware of doxycycline cardiotoxicity in veal calves in North America.**

Although not approved for use in cattle in Canada, the doxycycline was legally imported as an "Active Pharmaceutical Ingredient". An important issue arising from this case, in addition to cardiotoxicity, is the importation of non-licensed antibiotics albeit under a legal mechanism, and the possible impact of metaphylactic use of doxycycline in food-producing animals on antimicrobial resistance. Most recently doxycycline has been one of the drugs recommended for the prophylactic treatment of anthrax in people in the USA.

Reference

1. Zeeuwen AA, et al. Doxycycline-vergiftiging bij witvleeskalveren. Tijdschr Diergeneeskd 1993; 118: 803.

* "metaphylaxis" is the use of antibiotics for the prevention of disease or the control of its spread through the treatment of both symptomatic and in-contact animals.

Diagnosis of malignant catarrhal fever in Ontario

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From January 1998 to mid-January 2002, 26 cases of malignant catarrhal fever (MCF) were diagnosed at the Animal Health Laboratory (AHL) and Ontario Veterinary College (OVC). Of these, 22 were from cattle, 2 were from bison, and 2 were from deer (red and Sika). Although a complete clinical history was not available in many cases, contact with sheep and/or goats was indicated in 8 of 26 herds, and suspect cases in herdmates in 6 of 26 herds.

Conjunctivitis or uveitis was noted in 15 cases, gastrointestinal signs and/or lesions (resembling BVD) in 19 cases, respiratory signs and/or lesions in 13 cases, central nervous system signs and/or lesions in 19 cases, and systemic lesions in 23 cases.

In North America, MCF is caused by a herpesvirus and causes severe clinical disease with almost 100% mortality in most ruminants (except sheep, goats and fallow deer). The virus is relatively unstable in the environment but aerogenous transmission up to a kilometer has been suspected in some cases. It is not transmitted among clinically affected animals and is thought to be contacted from clinically normal sheep or goats, but this cannot be demonstrated in many cases and cases can occur several months after initial contact. Deer are not considered to be significant in the transmission of MCF.

In animals without ocular involvement, MCF can be very difficult to differentiate from BVD or IBR, and diagnosis is based upon characteristic histologic lesions of vasculitis and lymphoid hyperplasia.

In animals without ocular involvement, MCF can be very difficult to differentiate from BVD or IBR and diagnosis is based upon characteristic histologic lesions of vasculitis and lymphoid hyperplasia. Although rinderpest can cause similar clinical signs and gross/microscopic lesions, this virus is very unstable and unlikely to be introduced into North America.

At an extra cost of $105, PCR on blood and fresh tissue can be used for diagnosis. In submissions for this and other diseases, **it is important to have frozen tissues available for additional virology, bacteriology, toxicology or other tests if needed**; these can be kept at the clinic or submitted to the laboratory with a note "please hold frozen tissue for additional tests if indicated." Serology is not useful for screening sheep or goats for carrier status; contacts of sheep and goats with other ruminants should be avoided whenever possible.

Antimicrobial susceptibility testing…..do you need it for *Staphylococcus aureus* mastitis?

Marie Archambault, Beverly McEwen *marchamb@lsd.uoguelph.ca*

The AHL mastitis laboratory performed 43,691 procedures in 2000. These include milk and bulk tank milk cultures, somatic cell count (SCC), California mastitis test (CMT) and antimicrobial susceptibility testing (AST). The three most frequent isolates in the year 2000 were *Staphylococcus aureus*, *Streptococcus* non-*agalactiae* and *Escherichia coli* (Table 1). We perform AST according to the NCCLS guidelines using the *in vitro* disk diffusion method, also named Kirby-Bauer.

Antimicrobial susceptibility testing *in vitro* produces profiles that sometimes do not correlate with the *in vivo* outcome. For example, *S. aureus* is very susceptible *in vitro*, yet it is difficult to clear the infection. Interestingly, our data have shown that *S. aureus* mastitis agents rarely acquire resistance to commonly used antimicrobial drugs and are highly susceptible *in vitro* to a wide range of antimicrobial agents. *S. aureus* was susceptible *in vitro* to trimethoprim-sulfa (100%), cephalothin (99.9%), penicillin/novobiocin (99.8%), neomycin (99.6%), oxacillin (99.1%), clindamycin (97.3%), erythromycin (97.0%), pirlimycin (96.8%) and tetracycline (95%). However, the *S. aureus* cure rate is low because the microorganism forms microabscesses that drugs cannot penetrate. The bacteria can also survive within neutrophils where antimicrobial agents cannot reach them. *S. aureus* can also mutate into L-forms (bacteria without a cell wall that resists $-lactam antibiotics).

Since the *in vitro* laboratory AST for *S. aureus* will likely not correlate with the cure rate, *S. aureus* AST are no longer done routinely at the AHL laboratory. They will only be performed at the request of the veterinarian. This modification in our procedure reflects the fact that even though *S. aureus* is very susceptible on AST, and although mastitis products are labeled to be effective against *S. aureus* mastitis, the cure rate is low and should generally be considered negligible. The literature states that first-lactation cows with recent infections are most likely to benefit from antimicrobial therapy because of less extensive microabscess formation than in older cows. In addition, parenteral treatment in conjunction with intramammary therapy may increase the probability of effecting a cure. For our surveillance program, we will continue to perform AST on a subset of *S. aureus* isolates submitted to our laboratory. We will continue to monitor possible antimicrobial resistance changes of this bacterium.

(continued on next page)

Streptococcus non-*agalactiae* are variable in their antimicrobial resistance profiles according to *in vitro* data. The grouping of several different *Streptococcus* species under the name '*Streptococcus* non-*agalactiae*' probably contributes to the variability. Susceptibility testing in our lab demonstrates that *Streptococcus* non-*agalactiae* are most reliably susceptible *in vitro* to penicillin/novobiocin (99%), ampicillin (96%), penicillin (96%), cephalothin (92%), and erythromycin (90%). *Escherichia coli* were susceptible *in vitro* to trimethoprim-sulfa (90.8%), ampicillin (79.7%) tetracycline (71.5%), and cephalothin (71.3%).

The susceptibility profiles of *E. coli*, *S. aureus* and *S.* non-*agalactiae* appear to have remained quite stable over time when compared to the profiles obtained in 1997-1998 (AHL Newsletter 1998; 2(3): 5).

Table 1. Isolates from milk from across Ontario, AHL mastitis laboratory, Jan - Dec 2000

Microorganisms	**Number of isolates**
Staphylococcus aureus	5,258
Streptococcus non-*agalactiae*	3,910
Escherichia coli	1,959
Klebsiella spp	671
Streptococcus agalactiae	424
Arcanobacterium pyogenes	249
Prototheca spp	155

Since the *in vitro* laboratory antimicrobial susceptibility testing for *S. aureus* will likely not correlate with the cure rate, *S. aureus* AST is no longer done routinely at the AHL. AST's will only be performed at the request of the veterinarian.

In addition to *E. coli* and *S.* non-*agalactiae*, other environmental mastitis microorganisms include *Prototheca* spp. and *Arcanobacterium pyogenes*.

Prototheca spp. are algae that can cause chronic mastitis. The organism is ubiquitous in nature; consequently, they are common isolates from animal environments. Treatment is usually unsuccessful. *Prototheca* spp. can be introduced via intramammary infusion.

Arcanobacterium pyogenes, formerly *Actinomyces (Corynebacterium) pyogenes*, can cause sporadic cases of suppurative mastitis, referred to as 'pyogenes mastitis'. These cases usually respond poorly to treatment and the affected quarter is frequently lost for milk production. *Arcanobacterium pyogenes* isolates are susceptible *in vitro* to penicillin G and other $-lactam antimicrobials. However, failure of treatment can occur due to the purulent process in the gland, as antimicrobial distribution throughout the inflamed gland is limited. Even with intensive therapy, at least 80% of quarters are rendered functionless and many of those which respond are greatly reduced in productivity.

POULTRY

Genotyping of infectious bronchitis virus isolates from Ontario

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

Antigenic diversity of infectious bronchitis virus (IBV) is generated by emergence of "variant" viruses that sometimes "break" through vaccine-induced protection, even in properly vaccinated birds. IBV's were isolated from many Ontario clinical submissions received by our laboratory, but the identity (genotype/serotype) of these field isolates was unclear. Twelve Ontario field IBV isolates were therefore genotyped by phylogenetic analysis, a new molecular diagnostic method offered by the AHL. Since the spike (S) protein contains the major immunogenic portions of the virus, the S protein sequences from IBV's isolated in Ontario were compared to reference IBV serotypes to examine their degree of relatedness (Table 1). As a reference value, it is generally believed that the percentage of identity between viruses that belong to the same genotype/serotype will be at least 90%. *(continued on next page)*

Table 1. Percentages of identity of S protein sequences between Ontario IBV's, labeled **F**(ield) **I**(solate) and reference IBV serotypes.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
1		64.2	39.3	66.9	38.3	79.8	82.3	64.0	37.5	37.8	36.8	74.9	67.6	75.7	36.5	92.6	78.2	81.2	76.8	**1**	CAL-99
2	36.3		42.1	81.8	37.1	65.8	65.3	98.9	37.5	35.5	36.8	57.4	82.2	56.6	35.9	61.4	60.0	60.5	56.3	**2**	Conn
3	93.8	81.3		40.3	33.7	41.4	40.8	39.3	92.0	32.6	92.0	37.7	41.1	37.0	32.4	36.5	37.7	37.7	33.7	**3**	DE072-92
4	36.1	16.4	83.0		34.9	64.1	65.2	83.7	39.8	34.3	39.7	61.9	100.0	60.2	34.1	66.9	65.2	64.6	61.9	**4**	MA-5
5	94.8	90.9	109.7	92.7		36.6	34.3	37.1	32.0	96.5	30.5	35.4	35.4	34.9	97.6	37.7	36.6	36.6	34.9	**5**	PA-1220_98
6	22.5	37.7	90.3	42.0	100.4		92.5	64.0	36.9	34.9	36.2	84.8	64.9	85.2	34.1	81.5	92.7	93.8	91.1	**6**	PP14
7	19.1	38.5	90.3	40.1	104.4	7.9		63.5	36.4	33.7	35.6	84.8	65.9	84.1	32.4	83.1	91.7	93.2	88.9	**7**	Ark 99
8	37.8	1.1	88.9	15.7	90.1	40.1	41.0		37.5	35.5	36.8	61.2	84.3	59.6	35.9	65.2	64.0	63.5	60.7	**8**	FI-47
9	101.3	90.9	8.4	82.2	111.5	103.2	103.2	90.9		31.4	98.3	38.1	39.8	37.5	30.6	38.6	38.1	37.5	34.1	**9**	FI-25
10	99.9	95.8	113.8	96.7	3.6	108.0	110.2	95.0	115.2		30.2	35.5	34.3	34.9	96.5	37.8	35.5	35.5	33.7	**10**	FI-28
11	101.8	90.2	7.9	81.5	114.8	102.7	102.7	90.2	0.6	116.3		37.4	39.7	36.8	31.2	37.9	37.4	36.8	33.3	**11**	FI-31
12	28.7	46.3	96.8	43.3	102.4	15.7	16.4	44.3	98.5	105.9	97.9		61.6	95.8	34.7	76.7	88.5	88.5	84.7	**12**	FI-34
13	35.1	16.0	81.4	0.0	92.5	40.8	39.0	15.6	82.2	97.6	81.5	43.9		60.5	34.1	65.4	64.3	65.4	61.1	**13**	FI-35
14	28.9	48.0	98.9	45.6	105.6	15.9	17.9	46.7	100.4	109.3	99.9	4.4	45.6		34.1	75.7	87.3	88.4	84.7	**14**	FI-40
15	99.3	94.1	114.3	95.8	1.8	106.3	108.4	94.1	118.3	2.4	115.8	104.2	95.8	108.4		36.5	34.1	34.1	32.4	**15**	FI-18
16	6.2	37.3	98.9	37.4	100.7	21.3	19.2	36.6	101.3	105.0	100.7	26.0	37.4	26.9	104.2		82.5	83.1	79.4	**16**	FI-66
17	24.0	42.7	96.6	40.1	96.7	7.6	8.8	40.1	99.3	103.8	98.8	11.9	41.7	13.3	102.1	19.9		96.9	94.2	**17**	FI-43
18	20.5	41.1	95.9	41.1	96.7	6.5	7.1	41.0	101.3	103.8	100.7	11.9	39.9	12.7	102.1	18.7	3.2		94.7	**18**	FI-44
19	23.8	44.6	100.7	43.6	99.6	7.2	9.6	43.6	105.2	107.1	104.7	13.4	43.3	13.5	106.3	21.5	3.8	3.2		**19**	FI-46
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**		

The results are also presented as a "phylogenetic tree" - the distance between the "branches" on the tree indicates the relatedness between the viruses on those branches. Closely related viruses will be on the same, or on a nearby, branch (Figure 1). Ontario IBV isolates could be divided into three general groups: i) vaccine-like viruses (FI-35 and FI-47), ii) isolates similar or related to variant IBV's described in the USA (FI-18, FI-28, FI-25, FI-31 and FI-66), and iii) Ontario-specific isolates (FI-34, FI-40, FI-43 and FI-46). **It is not known how these strains were introduced into Ontario, but it appears that, much as in the USA, IBV variants continue to pose a threat to both layer and broiler industries.**

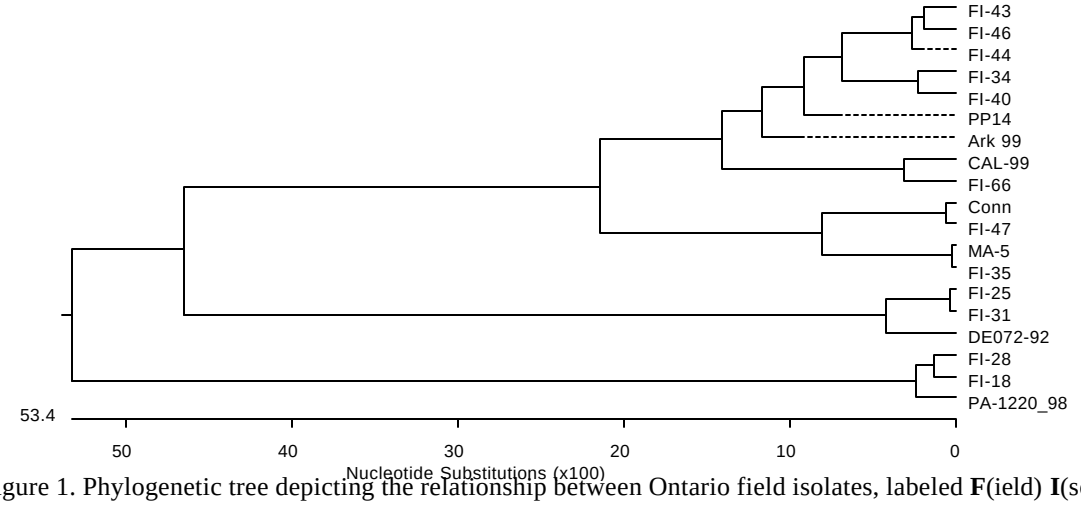

Figure 1. Phylogenetic tree depicting the relationship between Ontario field isolates, labeled **F**(ield) **I**(solate), and selected reference IBV serotypes.

Genotyping by phylogenetic analysis utilizes three techniques combined (reverse-transcriptase polymerase chain reaction, gene sequencing, sequence analysis) as the most advanced IBV diagnostic technique that is currently available. The test can differentiate between vaccine viruses and field isolates and allows the immediate comparison of field IBV variants with all previously isolated and characterized viruses.

This test is now offered by the AHL for a fee of $200.00. If you have any questions regarding this test, turn-around 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.

Reference:

Kingham BE, et al. Identification of avian infectious bronchitis virus by direct automated cycle sequencing of the S-1 gene. Avian Dis 2000; 44: 325-35.

SWINE

Actinobacillus pleuropneumoniae and *Actinobacillus suis* – an update

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Two members of the genus *Actinobacillus* are significant pathogens in pigs. *Actinobacillus pleuropneumoniae* is the etiologic agent of a severe necrotizing and hemorrhagic pleuropneumonia. *Actinobacillus suis* causes septicemia and death in suckling and recently weaned pigs, skin lesions resembling erysipelas in older pigs and sows, and a pleuropneumonia resembling that caused by *A. pleuropneumoniae*, in older pigs.

A. pleuropneumoniae has been divided into 12 serotypes (serotypes 1 and 5 are subdivided into 1a, 1b, 5a, and 5b), with serotypes 1, 5 and 7 being the most common in Ontario swine. The number of serotypes is likely to be higher as some isolates are untypable. Serotypes 1 and 5 are known to be among the more virulent serotypes. A review of the submissions from Jan. 1997 to Dec. 2001 to the Animal Health Laboratory identified the following serotypes from isolates (Table 1). The co-agglutination procedures used in identifying serotypes, were performed by Dr. Janet MacInnes, Department of Pathobiology, Ontario Veterinary College, University of Guelph.

Table 1. *A. pleuropneumoniae* serotypes identified by AHL

Serotypes	No. of isolates
1	37
1, 5*	5
1, 5, 7*	3
1, 7*	4
5	42
5, 7*	3
Untypable	8
Untyped	30
TOTAL	160

* Cross-reaction

These isolates came from 160 submissions from 114 different producers. *A. pleuropneumoniae* is particularly susceptible *in vitro* to many antimicrobials, but in clinically affected animals, therapy is effective only in the initial phases of the disease. A review of antimicrobial susceptibility testing (AST) revealed the following information (Table 2).

Table 2. AST pattern of *A. pleuropneumoniae*

Antimicrobial	No. tested	% susceptible
Ampicillin	118	72.0
Apramycin	76	39.5
Clindamycin	82	9.75
Ceftiofur	85	87.1
Gentamicin	113	71.7
Neomycin	81	65.4
Penicillin	116	37.9
Spectinomycin	78	42.3
Tetracycline	118	37.3
Tilmicosin	62	75.8
Trimethoprim-sulfa	117	66.7

Of particular interest were some of the susceptibility patterns of individual isolates. Seven isolates were resistant to all, eleven were resistant to all but one (6 were susceptible only to ampicillin, 2 each to apramycin and trimethoprim-sulfa, and 1 to gentamicin), and seven were resistant to all but two (3 were susceptible to only ampicillin and apramycin, 2 to ampicillin and tetracycline, and 1 each to ceftiofur and gentamicin and to ceftiofur and spectinomycin) of the antimicrobials in the panel. At our laboratory, AST is performed according to the NCCLS guidelines using the *in vitro* disk diffusion method, also named Kirby-Bauer.

One hundred and forty-six producers made 199 submissions of carcasses/specimens over a similar period of time, from which *A. suis* was isolated (Table 3). Interestingly, only five producers submitting pigs from which *A. suis* was isolated had also submitted animals infected with *A. pleuropneumoniae*. **Several reports have suggested that herd infection with *A. suis* confers a degree of protection against *A. pleuropneumoniae* infection, and our laboratory data further adds support to this theory.**

(continued on next page)

Table 3. AST pattern of *Actinobacillus suis*

Antimicrobial	No. tested	% susceptible
Ampicillin	171	73.7
Apramycin	104	29.8
Clindamycin	102	4.9
Ceftiofur	142	91.5
Gentamicin	160	76.9
Neomycin	98	54.1
Penicillin	170	54.1
Spectinomycin	96	51.0
Tetracycline	178	50
Tilmicosin	77	61.0
Trimethoprim-sulfa	171	77.2

Eleven isolates were resistant to all antibiotics in the panel, four isolates were resistant to all but one antibiotic (2 were susceptible only to trimethoprim-sulfa, and 1 each to ceftiofur and tetracycline), four isolates were resistant to all but two antibiotics (2 to ceftiofur and spectinomycin, and 1 each to ceftiofur and trimethoprim-sulfa, and to gentamicin and trimethoprim-sulfa). Of the five producers who had cases with co-infections with both microorganisms, only one showed multidrug resistance to both isolates.

Among points to note are the high resistance of swine *A. pleuropneumoniae* and *A. suis* to apramycin, clindamycin and tetracycline, and the high prevalence of susceptibility of these isolates to ceftiofur. Lincomycin has one derivative, clindamycin, and both have similar mechanisms of action. Lincomycin has been used extensively in the swine industry in the prevention and treatment of both *Mycoplasma hyopneumoniae* and *Brachyspira (Serpulina)* infections. This might help to explain the high degree of resistance to clindamycin noted in this study.

Among points to note are the high resistance of swine *A. pleuropneumoniae* and *A. suis* to apramycin, clindamycin and tetracycline, and the high prevalence of susceptibility of these isolates to ceftiofur.

Selection of an antimicrobial for treatment of *A. pleuropneumoniae* should be based on AST results because resistance has been documented for tetracycline, ß-lactam, sulfonamides, gentamicin, streptomycin, erythromycin, tiamulin, and tilmicosin. The most active antimicrobials are the cephalosporins. Irregular efficacy is noted with penicillin, tetracyclines, sulfonamides, and trimethoprim-sulfa.

Reference

Prescott, JF, Baggot, JD, Walker, RD. Antimicrobial Therapy in Veterinary Medicine. 3rd ed., 2000, Iowa State University Press.

HORSES

Crossiella equi placentitis in mares: Not in Ontario, eh?

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Recent press releases from Kentucky regarding *Crossiella equi* abortions in mares may result in questions from horse owners about this disease. Although the name of this microorganism is new, the disease is not. This entity was previously termed nocardioform placentitis, as the microorganisms isolated resembled *Nocardia* spp, of the Actinomycetales order of bacteria. Recent genetic analysis has placed this microorganism in the genus *Crossiella*. Placental infection with this gram-positive filamentous bacterium results in the distinctive lesion of localized placentitis at the junction of the uterine horn and body. This is very different from the usual cervical distribution of ascending placentitis in mares. Affected placenta is thickened and covered by abundant mucopurulent debris.

The pathogenesis leading to this unusual lesion localization remains unknown, but is thought to represent opportunistic infection by this environmental, soil-dwelling microorganism. In addition to late-term abortions, *C. equi* placentitis can result in weak or stillborn foals.

Weather conditions in the south-central United States may have influenced the recent increase in *C. equi* placentitis seen in Kentucky. To date, placentitis due to this bacterium has not been confirmed outside of that region.

A single case of suspected nocardioform placentitis was identified at the Animal Health Laboratory in 2000. The mare had been in the US for the majority of her pregnancy, and returned to Canada for foaling but aborted late in gestation. Gram-positive filamentous bacteria consistent with *C. equi / Nocardia* spp were identified in histologic sections of placenta; unfortunately, fresh placenta was not submitted for bacterial culture, so confirmation of the diagnosis was not possible.

Although *C. equi* placentitis has not yet been diagnosed in resident Ontario mares, we continue to evaluate equine abortion submissions for possible involvement of this bacterium.

Reference

Hong CB, et al. Etiology and pathology of equine placentitis. J Vet Diagn Invest 1993; 5: 56-63.

COMPANION ANIMALS

Canarypox – an important disease in aviary management

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Recently at the AHL there have been cases of high morbidity and mortality in imported canaries that were diagnosed with canarypox. This virus is the most lethal member of the Poxviridae family. Canaries exhibit the cutaneous (dry pox), diphtheritic (wet pox), or systemic forms of the disease, the systemic form being the most common.

The incubation period ranges from 4 days to 3 weeks and any age group is susceptible. Clinically, birds show acute onset of dyspnea, followed by death due to pneumonia in >70% within 1-3 days. The chronic form results in proliferative dermal lesions around the eyes, mouth, nostrils or feet. Flock mortality can vary from 20-100%, depending on virus strain, location of lesions and susceptibility of the individual. The virus is most commonly transmitted by latently infected birds through direct contact between birds when fighting, by biting arthropods (i.e. mosquitoes) resulting in disease being most prominent in the autumn and winter, or iatrogenically through intramuscular inoculation. Infections can spread rapidly through susceptible populations. The virus can be latent for years and reactivate due to a variety of stressors.

Canaries exhibit the cutaneous (dry pox), diphtheritic (wet pox), or systemic forms of the disease, the systemic form being the most common

On post mortem examination, small pneumonic foci and hemorrhages, fatty liver degeneration and jejunitis may be observed. On histology, Bollinger bodies (eosinophilic, intracytoplasmic inclusion bodies) can usually be noted in the skin, respiratory tract and oral cavity and are pathognomonic for this infection. However, inclusion bodies may not be seen in very acute cases making culture necessary for diagnosis. Virus may be intermittently shed in feces of asymptomatic carriers and may be identified by repeated fecal cultures.

Vaccination is reported to be the best control method (commercial attenuated, live) but provides only temporary immunity (maximum immunity 3-4 weeks post vaccination). Healthy canaries should be vaccinated every 6-12 months, preferably at fledgling and annually 1 month prior to expected arthropod exposure, but not within 4 weeks of egg laying. Vaccination can be done in the face of an outbreak and has been shown to decrease mortality in birds not yet showing clinical signs. The use of separate needles for each bird to prevent viral transmission during vaccination procedures is important.

References

1. Exotic and Laboratory Animals. In: The Merck Veterinary Manual, SE Aiello, ed., Merck & Co., Inc., Whitehouse Station, New Jersey. 1998: 1308.
2. Gerlach H. Viruses In: Avian Medicine: Principles and Application, Ritchie BW, Harrison GJ, Harrison LR, ed., Wingers Publishing, Inc., Lake Worth, Florida. 1994: 865-874.
3. Poxviridae. In: Avian Viruses: Function and Control, Ritchie BW, Wingers Publishing, Inc., Lake Worth, Florida. 1995: 302-304.

Submissions for canine *Leptospira* spp. serology increased, 1998 to 2001

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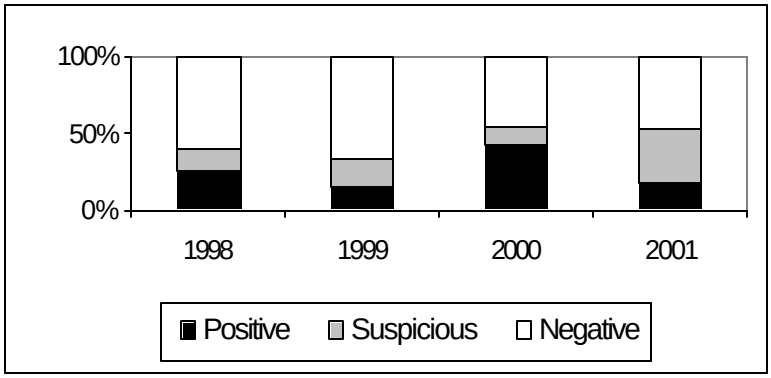
A greater than 5-fold increase in submissions for canine *Leptospira* spp. serology from Ontario dogs occurred in 2001 (n=213) compared to 1998 (n=42). While the combined frequency of seropositive (titers >320) and suspicious (titers of 80-160) cases did not change significantly from 2000 to 2001, **the proportion of seropositive cases decreased in 2001** (Figure 1).

Figure 1. Canine leptospira serology, microscopic agglutination test, 1998-2001.



Canine leptospirosis usually occurs seasonally, primarily in the fall, with sporadic cases in the spring. Last year differed from this pattern as cases occurred every month of the year, although the greatest number still occurred in the fall. Also, for the first time since 1998, *L. grippotyphosa* replaced *L. pomona* as one of the most frequent serovars identified (Table 1). Descending frequency of seropositivity in 2001 was to *L. autumnalis*, *L. bratislava*, *L. grippotyphosa*, *L. pomona*, *L. icterohaemorrhagiae*, and *L. canicola*. Further investigations and research of canine leptospirosis are ongoing at OVC and the AHL.

Table 1. Percent seropositive canine *Leptospira* spp. serovars, 1998 - 2001

	1998	**1999**	**2000**	**2001**
L. autumnalis	4.8	3.7	30.7	11.7
L. bratislava	16.7	7.4	24.8	10.8
L. grippotyphosa	14.3	1.9	15.0	9.4
L. pomona	16.7	3.7	26.1	6.1
L. icterohaemorrhagiae	0	5.6	13.7	3.8
L. canicola	0	1.9	0	1.4

Descending frequency of seropositivity in 2001 was to *L. autumnalis*, *L. bratislava*, *L. grippotyphosa*, *L. pomona*, *L. icterohaemorrhagiae*, and *L. canicola*.

When and how to use corticosteroid-induced alkaline phosphatase in practice

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Alkaline phosphatase (ALP) is a group of isoenzymes arising primarily from bone and liver. Elevated total ALP is seen in a variety of hepatic and cholestatic conditions including cholangiohepatitis and biliary obstruction. Dogs with endocrine diseases, including diabetes mellitus, hyperadrenocorticism, and hypothyroidism, often have a mild increase in alkaline phosphatase. **Corticosteroid-induced alkaline phosphatase** (CALP) is an isoenzyme of alkaline phosphatase found uniquely in dogs. It is produced in the liver in response to endogenous or exogenous corticosteroids, and requires at least one week before appearing in the peripheral blood.

Measuring CALP is a simple, inexpensive step that can be included in the veterinarian's arsenal of tests to rule out hyperadrenocorticism. **Its routine measurement is not useful due to the variety of conditions that can induce its elevation.** When interpreting an elevated CALP, it is vital to know if the dog has been exposed to any corticosteroids within the last 3 months. Dogs without evidence of hepatic disease, but with an elevated total alkaline phosphatase, can be tested for CALP. When CALP is greater than 100 IU/L or greater than 50% of total alkaline phosphatase, hyperadrenocorticism should be included in the list of differentials.

Rules for requesting and interpreting CALP

1. The dog has not been exposed to any medications with corticosteroids for the last 2-3 months including eye drops, skin creams, pills, or injections.
2. The dog has a moderately to marked increased alkaline phosphatase on routine biochemical profile.
3. The dog does not have other evidence of hepatic disease (clinical signs or biochemical abnormalities).

If the above 3 conditions are met, then:

4a. *an absolute value of CALP greater than 100 IU/L or a %CALP greater than 50%* warrants further investigation for hyperadrenocorticism (dexamethasone suppression test, ACTH stimulation test).

4b. *an absolute value of CALP less than 100 IU/L and a % CALP less than 50%* suggest that hyperadrenocorticism is unlikely to be the explanation for the elevated total ALP levels. Other disease conditions, especially mild hepatic conditions, are more likely and should be pursued.