UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

TAT's - helping the lab help you

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TAT's (turnaround times) are very important to you, the practitioner, as well as to us in the lab. In specimen reception, we set aside samples that require splitting until all other samples have been processed. There are several things that clinics can do to speed TAT's at the lab:

- Give us a **complete history**, telling us what tests you need - even if you aren't sure which test you want, please write out your needs on the submission form as completely as you can. This saves you and us from playing telephone tag.
- **Separate the serum from the clot** for Clin Path profiles - not only is the serum of better quality, but it can go directly into the analyzer instead of having to be spun down first.
- **Split serum according to the headings on the requisition**, e.g. check the Ruminant Submission Form AHL-6, front page SEROLOGY section - you will see headings for Mammalian Virology, Immunology, Mycoplasmology, and Sent to external labs. Separate serum samples are required for each of these lab sections. All of the tests listed require 0.5 mL of serum (width of baby fingernail); note 3 mL for respiratory panel.
- **Divide feces into separate containers** for Parasitology, Virology, and Bacteriology. Please use screw-cap vials NOT serum tubes, plastic lunch bags, or rectal sleeves. A large tablespoon-size of feces is usually sufficient.
- **Divide tissues according to the lab**, e.g. lung for BRSV FA, Virus isolation, Mycoplasma culture, and Bacterial culture should be divided into four pieces and packaged in separate Whirl-Paks, since these tests involve four different lab sections.

Please call the lab before submitting if you are unsure of the correct sample needed.

Testing of horses for West Nile virus in 2002

Susy Carman *scarman@lsd.uoguelph.ca*

In the summer of 2001, West Nile virus (WNV) was identified in wild crows and blue jays in many counties in southwestern Ontario. Horses can be seriously affected by WNV, and exhibit signs of neurological disease. The differential diagnosis of neurological disease in horses in Ontario must include rabies, with such cases being referred to the Canadian Food Inspection Agency (CFIA) for rabies virus testing. The CFIA will also collect specimens for WNV testing. **Testing for WNV at the National Centre for Foreign Animal Disease Laboratory in Winnipeg will commence after the horse has been shown to be rabies negative.**

Not all horses die from WNV infection. **A serological diagnosis can be made using paired sera in serological assays to identify a significant rise in antibody titer.** For this testing, sera can be forwarded by the AHL to the Cornell University Diagnostic Laboratory. The cost of testing using a serum virus neutralization assay is $25US per test. The cost of shipment of specimens to the US is $30 per case.

Information on surveillance for West Nile virus infection in wild birds can be obtained by contacting the Canadian Cooperative Wildlife Health Centre, Ontario Region at (519) 823-8800, ext. 4662; email at ccwhc@ovc.uoguelph.ca; or on the Web at http://wildlife.usask.ca

Why are oxacillin/methicillin-resistant *Staphylococcus aureus* important?

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Oxacillin/methicillin-resistant *Staphylococcus aureus* (MRSA) were identified in humans shortly after the introduction of methicillin into clinical practice in the early 1960s. MRSA are important pathogens because they are common causes of hospital-acquired infections, have limited treatment options, and are transmissible. A survey of 178 licensed human bacteriology laboratories in Ontario revealed that the total number of MRSA isolates reported in Ontario increased from 471 in 1992 to 1426 in 1995. Data from our laboratory indicate that MRSA have become established in our canine and equine populations. Routine screening procedures for identification of MRSA in a veterinary diagnostic bacteriology laboratory have become an important part of an adequate veterinary surveillance system.

In the last 2 years, 5 MRSA were isolated at the AHL bacteriology laboratory. Their antimicrobial susceptibility profiles were unpredictable and most were multi-resistant isolates (Table 1). The first MRSA was recovered in July 2000 from an incision swab of a Trakehner horse with an infected surgical site. The second MRSA was isolated from a Thoroughbred horse with an infected surgical site in January 2001. Three isolates were cultured in April and May 2002: from a septic carpal joint of a premature, catheterized foal; from a catheter tip from a Thoroughbred foal with thrombocytopenia; and from a Labrador retriever with marked septic suppurative inflammation in its stifle and hock joints.

Remember that **scrupulous handwashing** is always considered a good control measure!

MRSA can be carried by an individual animal or human as normal flora, usually on the skin or in the nose. MRSA is not more virulent than the more common *Staphylococcus aureus*. However, MRSA is of particular importance because if these infections occur they are very difficult to treat. Immunosuppressed animals or those with invasive devices such as catheters are much more likely to acquire MRSA infections. **The main mode of transmission of MRSA is via hands contaminated by contact with colonized or infected animals or humans, or with equipment and environmental surfaces.** Standard precautions, as described in the "Guideline for Isolation Precautions in Hospitals", include patient isolation, handwashing, gloving, masking, gowning, appropriate reusable equipment and laundry handling.

Fortunately, MRSA remains an infrequent isolate in our laboratory, and these infections are thought to be human in origin. However, veterinarians should carefully read bacteriology reports and look for 'MRSA' in the comment section. You can get more information on MRSA by consulting the Centers for Disease Control and Prevention (CDC) web site: http://www.cdc.gov/ncidod/hip/aresist/mrsa.htm or your local health department listed in the telephone directory.

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Oxacillin/methicillin-resistant *Staphylococcus aureus (continued)*

Table 1. Antimicrobial resistance patterns[1] of MRSA[2] isolates from Ontario, AHL bacteriology laboratory.

Antimicrobial agent	MRSA 1	MRSA 2	MRSA 3	MRSA 4	MRSA 5
Enrofloxacin	S	S	S	R	S
Erythromycin	R	NA	R	R	S
Gentamicin	R	R	R	R	S
Tetracycline	R	R	R	R	S
Trimethoprim-Sulfa	R	R	R	R	S
Amikacin	S	S	I	R	NA
Chloramphenicol	S	S	R	I	NA
Ciprofloxacin	S	S	S	R	NA
Tobramycin	R	I	R	R	NA
Rifampin	NA	NA	R	R	NA
Vancomycin[3]	NA	NA	R	NA	NA

NA = not available at this time. 1= Antimicrobial susceptibility testing is performed according to the NCCLS (M31-A) guidelines using the *in vitro* disk diffusion method. 2 = MRSA screening is performed according to the NCCLS (M100-S9) guidelines. If staphylococci are resistant to oxacillin, they should be considered resistant to all of the cephalosporins, amoxicillin-clavulanic acid, imipenem, ampicillin-sulbactam, or other B-lactam antimicrobials, regardless of *in vitro* results. 3 = Not routinely tested

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Chronic wasting disease testing now available for elk and deer

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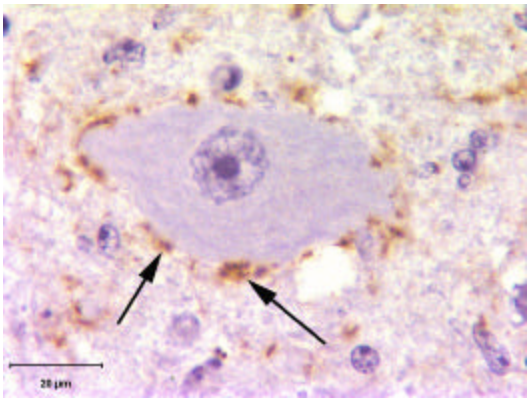
The AHL now has a specially built facility for the testing of some animal transmissible spongiform encephalopathies (TSE's), including chronic wasting disease (CWD) of cervids. **As with all current TSE tests, only a post-mortem test is available**. The test is an immunohistochemistry test using a monoclonal antibody (F99). Screening is done in Guelph, and if the test is suspicious, materials are forwarded to the CFIA Animal Disease Research Institute in Nepean, Ontario, which is the federal reference laboratory for this disease.

The fee for the test is $60. If three or more from the same location are submitted at the same time, the fee is $50 each per test. Proper sample collection is critical for this test, as the area of interest is the dorsal motor nucleus of the vagus nerve. This is conveniently located in the obex, which, with a small amount of training, is easily removed through the foramen magnum - the skull does not need to be opened. Training and a kit are available for interested veterinarians through Dr. Bob Wright at the Fergus OMAF office, phone (519) 846-3412. Owners may also bring heads directly to the AHL in Guelph or Kemptville where for $20 the obex will be removed and forwarded for testing. **Please do not send intact heads via mail or courier.** If the required 1.5 cm slice of obex is properly fixed 1:10 in formalin, turnaround time is 5 working days for a negative sample.

Dr. Aru Balachandran of ADRI strongly recommends additional collection of frozen tissue - brain just caudal to the fixed obex section would be appropriate, and can be held frozen in a labeled bag at the veterinary clinic until a negative test result is obtained. If a suspicious test result is obtained, this allows ADRI to do additional tests (Western blot) to confirm or rule-out CWD.



CWD-infected elk brain stained in our new lab. The dark granular material (arrows) is infectious prion.

AHL Lab Reports

CATTLE

Increased stillbirths and perinatal deaths in beef cattle

Peter Lusis *plusis@lsd.uoguelph.ca*

This spring, there have been reports of increased stillbirths and neonatal deaths (weak calf syndrome) in beef cattle in Canada and the US. The consensus on the AAVLD listserve is that **this is mostly due to fetal protein-energy malnutrition from under-conditioned beef cows because of poor crop conditions (drought, etc.) in the past year**. Similar problems have not been reported in dairy cattle, presumably because of better nutrition required to maintain milk production throughout pregnancy.

Other causes of stillbirths and neonatal death in cattle include:

- dystocia or perinatal trauma
- uterine inertia
- intrauterine infection
- hypothyroidism
- vitamin E-Se deficiency, and
- vitamin K deficiency.

Necropsies should be done on one or more calves:

- Are calves smaller than normal (fetal malnutrition, intrauterine infection)?
- Are fat stores minimal or absent (fetal malnutrition)?
- Are the thyroid glands enlarged (hypothyroidism)?
- Is there evidence of excessive placental or internal hemorrhage (birth trauma, vitamin K deficiency)?
- Are there lesions suggestive of infection?

As with all submissions, a detailed history, including vaccinations, is essential for diagnosis.

For **laboratory testing**, please submit

- for **histopathology -** brain, thyroid, lung, heart, skeletal muscle, liver, kidney, placenta
- for **bacteriology -** placenta, lung, stomach content
- for **mycoplasma** isolation - placenta, lung, stomach content
- for **virus** isolation - thymus, thyroid, placenta, lung, liver, spleen
- for **serology** - acute and convalescent **serum**

It is very important to **assess the body condition of cows in affected herds** - this can be difficult to do visually because undernourished beef cows often appear to be in good body condition due to long haircoats and abdomens distended with poor quality, indigestible roughage. First-calf heifers are especially susceptible to loss of condition during pregnancy.

High-protein forage (>10% crude protein) should be fed for at least 60 days before calving, and protein supplements are required if poor-quality hay is the major source of feed.

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2. Body condition scoring of beef cows. OMAF Factsheet, September, 1985.

Update on bovine viral diarrhea virus testing

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The Syracuse BVDV ELISA is the test of choice for the rapid identification of animals persistently infected with BVD virus. Serum or plasma is used for animals over 3 months of age. **Ear notches** or skin biopsies are used for animals under 3 months of age.

We also offer a PCR test for evaluation of serum, blood, and bulk milk tank samples for BVD virus.

For details please see the **May 1, 2002, AHL Lab Note #1, "Update on BVD virus testing at the Animal Health Laboratory**" on our website: http://ahl.uoguelph.ca

2001 - Update on bovine viral diarrhea virus typing at the AHL

Susy Carman, Beverly McEwen, Murray Hazlett, Dirk Deregt *scarman@lsd.uoguelph.ca*

Over the last 4 years, the AHL has recovered 661 bovine viral diarrhea virus (BVDV) isolates from various bovine specimens submitted for virus isolation. A detailed summary of isolates by year appears in the table below. Of the total of 161 isolates, 143 (21.6%) were cytopathic, being recovered from animals with mucosal disease. The 518 (78.4%) noncytopathic BVDV isolates were recovered from animals with various acute and chronic disease processes.

Prior to 2001, there had been fewer BVDV isolates recovered each year since the outbreak of severe acute BVD in Ontario in 1993-1995, with a gradual decline in cytopathic BVD isolates. However, for 2001 there is an increase in both the number of BVDV isolates and the proportion that are cytopathic (from 14.3% in 2000, to 28.8% in 2001). Most of this change is due to an increase in BVDV type 1 cytopathic isolates.

> Most of our increase in BVDV isolates in 2001 is due to increased numbers of BVDV type 1 cytopathic isolates.

Over these 4 years, we have continued to provide genotyping for most BVDV isolates, using either monoclonal antibodies or PCR genotyping. Overall, 328 (49.6%) isolates were determined to be BVDV type 1, while 322 (48.7%) were BVDV type 2. Prior to 2001, there had been a gradual decline in the proportion of all BVDV type 1 isolates, with a corresponding increase in the proportion of all BVDV type 2 isolates. That trend appears to have changed, with an increase in BVDV type 1 isolates (from 33.3% in 2000, to 54.1% in 2001).

BVDV type/year	**1998**	**1999**	**2000**	**2001**	**Total**
Total for each year	199	166	126	170	661
Total cytopathic	43 (21.6%)*	33 (19.9%)	18 (14.3%)	49 (28.9%)	143 (21.6%)
Total noncytopathic	156 (78.4%)	133 (80.1%)	108 (85.7%)	121(71.1%)	518 (78.4%)
BVDV type 1					
Cytopathic	26 (13.1%)	23 (13.9%)	7 (5.5%)	42 (34.7%)	98 (14.8%)
Noncytopathic	85 (42.7%)	60 (36.1%)	35 (27.8%)	50 (41.3%)	230 (34.8%)
Total	111 (55.8%)	83 (50.0%)	42 (33.3%)	92 (54.1%)	328 (49.6%)
BVDV type 2					
Cytopathic	15 (7.5%)	10 (6%)	11 (8.7%)	7 (0.6%)	43 (6.5%)
Noncytopathic	71 (35.7%)	73 (44%)	69 (54.8%)	66 (38.8%)	279 (42.2%)
Total	86 (43.2%)	83 (50.0%)	80 (63.5%)	73 (42.9%)	322 (48.7%)
BVD type 1-2 mixture	2 (1%)	0	0	1 (0.6%)	3 (0.5%)
Typing not attempted			4 (3.2%)	4 (2.4%)	8 (1.2%)

***** proportion of the total for each year in brackets

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POULTRY

Salmonella pullorum – Changes to export testing for "poultry"

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Pullorum disease (caused by *Salmonella pullorum* infection) is a reportable disease in Canada. Recent changes to the certification of poultry for export to the USA require *S. pullorum* testing. These changes were made by the CFIA after evaluation of the current document (CFIA 1513) and consultation with the USDA.

Flocks that are large enough to participate in the federal hatchery program are already tested for *S. pullorum.* However, for flocks of origin not participating in the federal hatchery program (i.e. waterfowl, game birds, backyard poultry, or "fancy birds"), there is now a specific requirement for *S. pullorum* testing to allow export to the USA.

For flocks of origin not participating in the federal hatchery program (i.e. waterfowl, game birds, backyard poultry, or "fancy birds"), there is now a specific requirement for *S. pullorum* testing to allow export to the USA

For purposes of certification for entry into the USA, poultry is defined as chickens, ducks, geese, swans, turkeys, doves, pheasants, grouse, partridges, quail, guinea fowl, peafowl, and pigeons. The Animal Health Laboratory can facilitate testing for *S. pullorum* that involves sampling of the environment of the flock of origin within 90 days of export.

Based on the Ontario Hatchery and Supply Flock Policy, **the minimum number of environmental swabs required for a flock is 3 swabs or 1 composite swab per 1000 birds. Vials of peptone water, sterile swabs and sampling instructions can be obtained through the Animal Health Laboratory.**

The swabs should be run through waterers, dust (e.g. air intakes, waterlines, nests), litter and fresh fecal material. Testing should be done in sequence from the cleaner to the more contaminated sites. Refrigerate samples and send to the AHL with a submission form clearly marked "For *Salmonella pullorum* export testing – Poultry Bacteriology". The fee for this testing is $14.00 per swab.

Turnaround time for initial AHL bacteriology is approximately 7 days. If *Salmonella* is isolated, then the sample is sent to Health Canada for serotyping and phage typing, which takes a further 2 weeks (i.e. approximately 21 days if *Salmonella* is isolated on initial culture).

For further sampling information, please contact either Keith Harron (ext. 4543, kharron@lsd.uoguelph.ca) or Jacquie Caccavella (ext. 4528) at the Animal Health Laboratory.

For further information regarding requirements for poultry export to the USA, accredited veterinarians should contact their local CFIA district veterinarians. For a list of offices and contact information for local district veterinarians, please refer to the CFIA website at: www.inspection.gc.ca/english/anima/heasan/offbure.shtml .

Infectious laryngotracheitis—diagnosis, reporting and communication

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Confirmation of diagnosis, disease management, and communication are important components of managing an outbreak of infectious laryngotracheitis (ILT). Infection with the ILT herpesvirus can cause acute respiratory disease in chickens. Mild and acute forms exist, with clinical signs ranging from watery eyes, nasal discharge and swollen sinuses, to coughing and bloody exudate from the trachea. The economic impact of ILT results from decreased egg production (5-15%), decreased growth rates, and the impact on export markets.

Infectious laryngotracheitis, chlamydiosis, fowl cholera, and avian encephalomyelitis are diseases for which certain Canadian trading partners (e.g. Russia and China) require special notification. For the short term, all segments of the poultry industry have reached a national consensus with federal and provincial governments on an interim system of reporting these diseases to permit trade to continue.

This system starts with the voluntary submission of a case to the Animal Health Laboratory by a veterinarian. If the case is considered suspicious for any of these four avian diseases, the referring veterinarian is contacted for further information (i.e. more flock history, where the flock is to be processed, etc.). For ILT, confirmation may be based on histological evaluation (larynx, trachea, lung sections), however virus isolation will also be attempted at the AHL on appropriate samples (fresh trachea and lung).

Infectious laryngotracheitis, chlamydiosis, fowl cholera, and avian encephalomyelitis are diseases for which certain Canadian trading partners (e.g. Russia and China) require special notification

The Provincial Veterinarian, Ontario Ministry of Agriculture and Food, is notified by the AHL veterinarians, and general flock history and information on processing location (i.e. provincial vs. federal) is communicated if known. The Provincial Veterinarian, referring veterinarian, and the producer discuss the disease, preventive steps, where the birds will be processed, and the potential for export.

Canadian Food Inspection Agency (CFIA) - Ontario Region veterinarians are notified with the permission of the producer and based on a waiver of confidentiality signed by the producer. All segments of the national poultry industry involved with export of poultry products requested their producers to sign these waivers. They also requested producers to record the diagnosis of these four diseases on flock health sheets. There is no penalty for these flocks and they are safely processed for non-export markets. Usually the flock is moved to the end of the processing line to allow segregation of the final product and to allow thorough cleaning of the facility after processing.

Biosecurity is key to preventing the risk of ILT and other contagious poultry diseases. This includes communication about biosecurity with feed companies, rendering companies, catching crews, trucking companies and other personnel in contact with the flock to minimize the occurrence of the infection. Natural or vaccine exposure to ILT virus can produce latent carriers, and birds can intermittently shed the virus if stressed. Any naïve birds will be susceptible to infection and the organism can be transmitted to other facilities on clothing, vehicles and other mechanical vectors. ILTV is a relatively fragile virus, and thorough cleaning, disinfection and down-time will provide a good basis for eradication. Operations with multi-age flocks are at higher risk of exposure despite vigilant biosecurity. Veterinarians may also use vaccination to minimize the impact of an outbreak in some cases. Timely communication and strict biosecurity are key to prevent and control the spread of ILT.

This interim approach of reporting ILT is a workable system to keep export markets open. In Ontario, it uses an expert surveillance network involving the AHL, OMAF, the CFIA, and the initiative of the poultry industry. However, a long-term solution is to make these four poultry diseases reportable under the federal Health of Animals Act. This move is supported by national poultry organizations, and discussions with the CFIA are ongoing.

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SWINE

Mycoplasma hyopneumoniae lung tissue PCR is available at the AHL

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The AHL has developed and validated a rapid, sensitive and specific polymerase chain reaction (PCR) method for the detection of *M. hyopneumoniae* in pig lung tissues, and will offer this test beginning May 1, 2002.

Advantages of using a PCR test for *M. hyopneumoniae* diagnosis include high specificity, sensitivity and rapid turn-around of test results. The test can identify evidence of infection in vaccinated herds, unlike the serum ELISA that cannot differentiate between field exposure and vaccination. A PCR method has been reported for detection of *M. hyopneumoniae* in nasal swabs by amplifying the 16S rRNA gene. We have adapted and validated this PCR assay for the detection of *M. hyopneumoniae* from lung tissues.

The results of validation showed that the *M. hyopneumoniae* PCR test is a rapid, sensitive and specific diagnostic method for detection of *M. hyopneumoniae* in swine lung tissues. The analytical sensitivity of the test on lung tissue spiked with *M. hyopneumoniae* is 0.18 cfu/g. It is specific, with no false positives on 36 strains of different non-hyopneumoniae *Mycoplasma* spp. and other commonly isolated bacterial strains of 23 species. There were no false negatives on any of the 8 strains of *M. hyopneumoniae* tested. The PCR test agreed substantially (kappa= 0.66) with the fluorescent antibody test, currently the most sensitive diagnostic method. Therefore, this PCR assay is suitable for clinical diagnosis for detecting *M. hyopneumoniae* on lung tissue samples.

Please submit a small piece of pneumonic lung tissue from the edge of the lesion, frozen and packed with freezer packs, and indicate on the submission sheet that the *M. hyopneumoniae* PCR test is requested. The fee for the test is $16/sample.

The *M. hyopneumoniae* PCR test is a rapid, sensitive and specific diagnostic method for detection of *M. hyopneumoniae* in swine lung tissues.

Due to the complex nature of respiratory disease in swine, with multiple pathogens often occurring in the same animal, we recommend that you also submit pneumonic lung tissues for histology, bacteriology and virology examination (see AHL User's Guide, pp 41, 42). As with other tests, inappropriate sampling and other factors may cause false-negative results. Therefore, a negative result should be interpreted with caution.

Acknowledgment

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The cost of not obtaining an accurate diagnosis

Reg Reed, Gaylan Josephson *gjosephs@lsd.uoguelph.ca*

Piglets from a newly established, 500-sow breeding herd were weaned at 17-19 days of age into nurseries in a three-site production system. The usual new startup herd health problems, e.g. greasy pig disease, reproduction problems, etc., were handled successfully. Reproduction parameters for a 12-month period were 2.4 litters/sow/year, with 10.5 piglets born alive per litter. This resulted in an average of 21.16 piglets weaned per sow per year.

However, a persistent piglet diarrhea problem affected piglets between 4-15 days of age, and the resulting preweaning mortality averaged 16%. Rectal swabs were submitted to the AHL diagnostic laboratory and an enterotoxigenic strain of *Escherichia coli* was isolated.

Discussions among the owner, farm staff and the herd veterinarian led to several recommendations. Routine management changes, e.g. sow vaccination, improved sanitation, etc., did not appreciably improve piglet performance. Other issues including feed, water, piglet chilling, and piglet processing were investigated, but deficiencies or problem areas could not be identified.

The veterinary practitioner strongly recommended that affected piglets be submitted to the laboratory for a complete diagnostic workup. Because of the time involved (the farm was located 2 hours from the laboratory), the direct costs related to laboratory testing, and the perceived lack of benefit based on the results from the initial submission of rectal swabs, the producer initially did not follow this advice. However, after struggling with the problem for more than one year, 3 live, recently affected, scouring piglets were submitted to the AHL, and a rotavirus infection was identified in sections of intestine, using the fluorescent antibody technique. A pre-farrowing vaccination program was initiated immediately. The performance of rotavirus vaccines in various studies has resulted in conflicting data, but a significant and positive response to sow vaccination, identified as a significant reduction in severity of piglet diarrhea with a resulting reduction in mortality and an increase in weight gain, was recorded in this herd.

The lost income related to the rotavirus diarrhea problem was calculated to be $35,200 over a one-year period, again proving the value of identifying the specific cause and taking appropriate corrective action.

The lost income related to the rotavirus diarrhea problem was calculated to be $35,200 over a one-year period, again proving the value of identifying the specific cause and taking appropriate corrective action.

Most production-limiting health problems can be dealt with, although the ease with which they are recognized, diagnosed and resolved may vary widely. Choosing to ignore any of these issues can be costly. Once recognized, the issue must be thoroughly investigated, a correct diagnosis made, and proper intervention strategies implemented.

Reference

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Table 1. Production parameters for 12-month periods, prior to the rotavirus diagnosis being made and after initiation of sow rotavirus vaccination.

	Before rotavirus diagnosis	After diagnosis & correct intervention
No of pigs born alive/litter	10.5	10.5
Litters/sow/year	2.4	2.4
Preweaning mortality	16%	11%
Piglets weaned/litter	8.8	9.34
No. of farrowings	1203	1201
Piglets weaned/12 months	10,584	11,215
% of no. 1 pigs (total)	90% (9,525)	95% (10,654)
Value of no. 1 pigs @$40.00/pig	$381,000	$426,160
% of no. 2 pigs (total)	10% (1,058)	5% (560)
Value of no. 2 pigs @$20.00/pig	$21,160.00	$11,200
Value of piglets sold	$402,160.00	$437,360.00

HORSES

Larval cyathostomiasis in horses: a new disease in Ontario?

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Strongyle parasites are very commonly found in the large intestine of horses, and can cause disease that ranges from ill-thrift to sudden death. As a result, these parasites are the primary reason that horses should be maintained on a regular deworming program. Until approximately 20 years ago, the large strongyles (particularly *Strongylus vulgaris*) were considered the most important strongyles. However, subsequent to the introduction of ivermectin, the large strongyles have become relatively uncommon. In contrast, **the small strongyles (cyathostomes) are now generally considered the most important intestinal parasite of horses and produce the majority of strongyle eggs found in the feces of horses**. Cyathostomes are acquired by horses at pasture and, upon ingestion, undergo a period of migration in the mucosa/submucosa of the large intestine that lasts weeks to months and includes the formation of encysted larvae. Thereafter, the larvae emerge into the lumen of the gut to mature. Disease is primarily due to development of the larval (immature) stages in the wall of the gut.

Clinical signs that are typically associated with larval cyathostomiasis include weight loss, acute/chronic diarrhea (not present in all cases), pyrexia, subcutaneous edema and colic

Since 1998, there have been 10 cases of equids with larval cyathostomiasis submitted to post-mortem from the Veterinary Teaching Hospital (VTH) OVC (n=3) and external practitioners (n=7). Larval cyathostome infestation was determined to be the primary cause of clinical illness in 8/10 cases. A variety of breeds including Belgian, Quarter Horse, Thoroughbred, Standardbred, Welsh pony and a donkey were affected. Cases occurred between September to March and the median age was 17.5 months.

Diarrhea, colic and weight loss were common clinical signs, although one horse was found dead without premonitory signs. Hypoproteinemia and/or decreased glucose absorption were reported in horses submitted from the VTH. Gross lesions included colonic mucosal miliary to 1-cm nodules (n=5), or colonic mucosal hyperemia (n=2); several horses had no gross intestinal lesions. Microscopically, nematodes (cyathostomes) encysted within the colonic mucosa were consistently accompanied by edema, lymphoplasmacytic or granulomatous infiltrates (Figure 1).

Larval cyathostomiasis is associated with the sudden development of large numbers of cyathostome larvae in the wall of the large intestine. The disease is relatively common in many parts of Europe, and has a seasonal occurrence there that is very similar to the cases reported here. Likewise, the disease is most commonly observed in 1-3 year old horses, and can occur in animals on a regular deworming program. Clinical signs that are typically associated with larval cyathostomiasis include weight loss, acute/chronic diarrhea (not present in all cases), pyrexia, subcutaneous edema, and colic. **Hypoalbuminemia is almost always observed, and strongyle egg counts are often negative.** For treatment of clinical cases, only two drugs have efficacy against encysted cyathostomes: moxidectin at 400 µg/kg bodyweight, and fenbendazole at 10 mg/kg bodyweight sid for 5 days. However, treatment is often unrewarding.

Over the last two years there has been a significant increase in the number of cases of larval cyathostomiasis diagnosed at the AHL and OVC. Whether this reflects a true increase in the incidence, drug resistance in cyathostomes, or other causes, is unclear and currently under investigation. In the mean time, it is prudent to regularly monitor the efficacy of anthelmintic regimens, and to combine anthelmintic use with management strategies that minimize pasture burdens of cyathostome larvae.

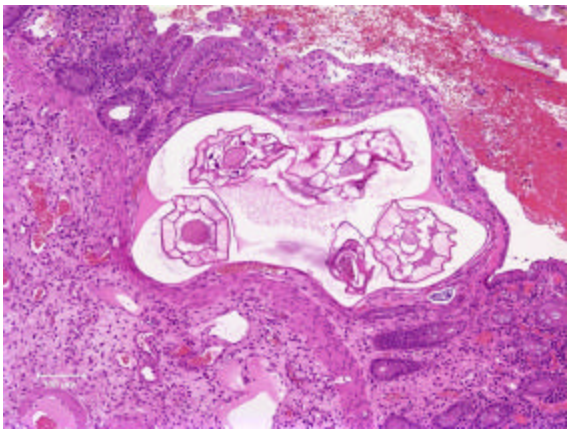

Figure 1. Cross sections of cyathostome larvae embedded in the colonic mucosa of a horse. Note hemorrhage in the gut lumen, upper right.

COMPANION ANIMALS

Nutritional osteodystrophy in puppies fed a BARF diet

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Nutritional bone disease in animals is now a rare and unexpected clinical entity as a result of our current knowledge of nutritional requirements and the availability of balanced, commercially prepared diets. A dangerous current trend promoting so-called **"BARF" diets** challenges sound research-based data on canine nutritional needs, and can result in severe and fatal osteodystrophy**. This acronym stands for "bones and raw food", or "biologically appropriate raw food",** and this homemade diet is composed of 60-75% raw meat and bones ground with some vegetables, eggs, or dairy products. Many Internet sites promote the diet, although no nutritional analyses or research basis for the diet are cited. The following unfortunate case illustrates the physical damage and suffering that such alternative diets can produce.

This unfortunate case illustrates the physical damage and suffering that such alternative diets can produce

Pups from 2 litters of 6-week-old large-breed dogs were presented because of hindlimb collapse, weakness, and failure to thrive. **The pups had been fed a BARF diet since 2.5 to 3 weeks of age**; one litter was weaned at the time of solid food introduction due to poor milk production by the bitch, and the second litter was weaned at 5 weeks of age. The dams had been fed the same diet during gestation.

All pups were weak, in pain, and were either unable to stand or had abnormal gaits. Two pups were radiographed, and all bones were poorly mineralized (osteopenic), with mid-shaft fracture or collapse in multiple long bones. In each of four pups euthanized and submitted for necropsy examination, all long bones were extremely pliable and cortices were very thin, allowing sagittal sectioning using only a scalpel blade. Parathyroid glands were prominent in all animals. The abnormal quality and quantity of bone were reflected histologically, where bone spicules were few, thin, and poorly mineralized, and cortical bone was often replaced by fibrous tissue. Despite these severe bone abnormalities, all pups had good internal fat stores and muscle mass. Calcium and phosphorus analyses on bone and on the complete diet are currently underway.

The lesions in these pups were consistent with nutritional secondary hyperparathyroidism and fibrous osteodystrophy resulting from an abnormal dietary calcium to phosphorus (Ca:P) ratio and likely also absolute calcium deficiency. Diets with an abnormally high meat content have Ca:P ratios ranging from 1:20 to 1:50, whereas the ideal ratio is 1:1. Secondary hyperparathyroidism due to the resulting low circulating calcium levels promotes osteoclast activity and resorption of bone, among other mechanisms designed to restore serum calcium to normal levels.

Rickets is an additional consideration in nutritional bone disease in young animals, and reflects defective ossification at growth plates due to vitamin D and/or phosphorus deficiency; however, growth plates in these pups had a histologically normal appearance. The rapidity of onset of debilitating bone disease in this case is remarkable; this likely reflects the rapid growth and bone development normally occurring in pups at this age, minimal bone mineral reserves to draw on initially in such young animals, and possibly defective prenatal bone mineralization due to the similarly poor quality of the dams' gestational diet.

Practitioners can use this example to counsel dog owners about the dangers of improperly balanced diets. An information sheet on BARF diets is available from the CVMA and will soon be published on their website (www.canadianveterinarians.net).

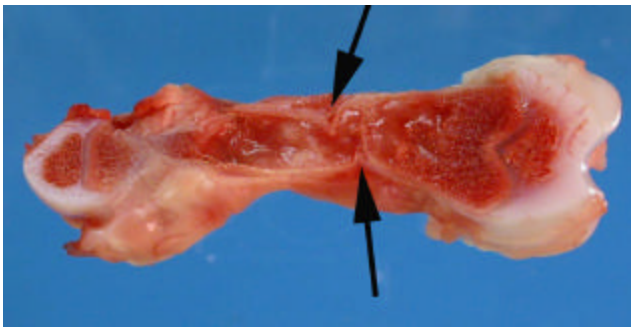

Figure 1. Femur from pup with nutritional osteodystrophy; bilateral midshaft cortical collapse (arrows) due to extensive bone resorption and fibrous replacement.

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Anticoagulant rodenticide poisoning and a mild Ontario winter

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Anticoagulant rodenticides are the second most common fatal toxicosis reported to North American poison control centers. Pesticides account for 25 to 30 % of calls about dogs and cats. The overwhelming majority of calls to poison control centers involve either insecticides or rodenticide products. The majority of pet deaths reported involved anticoagulant rodenticide products.

Anticoagulant rodenticide exposures and toxicosis occur most frequently in summer and early autumn, when owners distribute bait. We had a mild winter in Ontario, and rodents may have had a high survival rate this year, so there is likely to be more bait distributed.

The coagulopathy induced by anticoagulant rodenticides occurs because each of the compounds inhibits the "recycling" of vitamin K1. Clotting times increase as clotting factors are depleted. There are nine different rodenticide compounds on the North American market. They may be obtained over-the-counter or through pest control operators. Each compound is marketed in a consistent bait concentration, so the toxic dose can be presented as grams of bait per kilogram of pet.

The compounds most commonly sold in Ontario include: warfarin, bromodiolone, brodifacoum, pindone, chlorphacinone and diphacinone. We suggest that you become familiar with the products sold in your area.

Animals may not be presented with hemorrhages. **Often dyspnea, lethargy, and anorexia may be the presenting clinical signs.** They may begin to hemorrhage from any traumatized site when the coagulation times become prolonged. **You may see melena, epistaxis, hematuria and hematomas.** On autopsy, massive intrapulmonary or intraabdominal hemorrhage is found.

Exposure to anticoagulant rodenticides is most commonly confirmed by measurement of the specific compound in stomach content, source material, or liver samples. Coagulation tests are most commonly used in clinical settings to support a diagnosis of anticoagulant rodenticide toxicosis. The coagulation tests used are prothrombin time (PT) and activated partial thromboplastin time (APTT). Continued prolongation of PT or APTT 12 to 24 hours after adequate vitamin K1 therapy, supports a diagnosis of anticoagulant toxicosis.

Exposure to anticoagulant rodenticides is most commonly confirmed by measurement of the specific compound in stomach content, source material, or liver samples.

Analytical methods are available to identify each anticoagulant compound. High-pressure liquid chromatography (HPLC) is available at the AHL, to identify the specific compound in order to institute treatment and establish the dose and length of vitamin K1 therapy.

Reference

Murphy MJ. A Field Guide to Common Animal Poisons. Ames, Iowa: Iowa State University Press. 2001.

Questions, comments, suggestions for the editor??

- Questions about services provided by the AHL?
- Comments on our new Newsletter format?
- Suggestions for topics to be covered in future editions of the Newsletter?

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