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LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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In this issue:

Listeriosis – perspective from the AHL

Murray Hazlett, Marina Brash, Durda Slavic, Jim Fairles

Listeriosis was (and still is) in the news these days, with the massive processed meat recall issued by CFIA and Maple Leaf Foods last summer. In humans, listeria may cause abortion, neonatal infections, meningitis, and gastroenteritis.

In a review of 201 AHL pathology diagnosis records since 1997, we saw disease caused by *Listeria monocytogenes* almost exclusively as meningitis, microabscesses in medulla oblongata, abortion, or septicemia (Table 1). Diagnosis of listeriosis in brain disease in ruminants is commonly based on microscopic lesions alone, these being very characteristic both in appearance and location. Diagnosis in other conditions usually requires bacterial culture. Often this organism can be grown easily if samples are not heavily contaminated, and will be seen as small hemolytic colonies by 24-48 hrs. It is able to grow over a wide range of temperatures (4 - 45°C). Enrichment techniques are frequently employed to facilitate recovery from contaminated samples.

Cattle were our most common submission (97 cases), followed by sheep and goats. The only other species seen with regularity were chinchillas. Other species involved were 2 foals and 1 sugar glider with septicemia, and 2 rabbits with metritis. *AHL*

Table 1. Pathology cases associated with *Listeria monocytogenes*

Species	Neurologic	Abortion	Septicemia	Other	Total
Bovine	73	23	1	0	**97**
Caprine	30	7	0	0	**37**
Ovine	47	0	1	1	**49**
Chinchilla	0	0	13	0	**13**
Other	0	0	3	2	**5**
Total	**150**	**30**	**18**	**3**	**201**

Parathyroid panels - MSU

Michigan State **now will only accept 1 mL of serum plus 1 mL of EDTA plasma (frozen)** as acceptable samples for their parathyroid panels. Both types of samples must be collected - there are no substitutes.

Sheep MV & goat CAE testing

- **Caprine CAE** (caprine arthritis-encephalitis) ELISA testing is done at the AHL - $7.50/test.
- **Sheep MV** (maedi-visna) testing is sent to Quebec for the CFIA MV ELISA, which is now being done by LEPAQ - $11.00/test.
- **Sheep MV** testing program / project to eradicate MV through **OSMA** (Ontario Sheep Marketing Agency), test is offered by LEPAQ currently for $2.50/test, **special form required**.

AHL Christmas hours, 2008

- Limited testing services 0900-1700, Dec 24, 29, 30, 31, Jan 2/09 in both Guelph and Kemptville.
- Emergency mammalian necropsy and Specimen Reception are available in the Guelph lab on weekends and holidays except for Christmas Day, Dec. 25 (lab is closed) - our specimen drop box is available 24/7.
- Please call if you would like to schedule testing over the holiday period.

Season's Greetings from the staff of the Animal Health Laboratory

Serum protein electrophoresis for evaluation of hyperproteinemia

Kristiina Ruotsalo

The finding of increased serum protein values within a biochemical profile is not uncommon. First, it is important to determine if the increase is *relative* or *absolute*. **Relative hyperproteinemia** is due to dehydration and should be accompanied by concurrent erythrocytosis (unless the animal is also anemic), with increases in both albumin and globulin concentrations. It is important to remember that only dehydration will cause hyperalbuminemia. **Absolute hyperproteinemia** is due to an increased globulin concentration only (hypergammaglobulinemia), accompanied occasionally by a mildly decreased albumin concentration.

Hypergammaglobulinemia may be further characterized by serum protein electrophoresis. This method involves the separation of serum proteins into distinct bands within an agarose gel based upon their size and charge. The resulting electrophoretogram allows determination of which serum protein fractions are altered.

Generally there are 6, distinct protein bands present. These bands are albumin, α-1, α-2, β-1, β-2, and γ globulins. The α and β globulin fractions include acute-phase proteins, proteins involved in lipid transport, and complement. Some immunoglobulins (specifically IgM and IgA) may migrate within the β-2 region. The γ-globulin region contains immunoglobulins, primarily IgG.

Typically, 2 patterns of increase are noted within the globulin fraction. While neither pattern is pathognomonic for specific diseases, the electrophoretic patterns, in association with other laboratory data and clinical findings, may help in further defining disease conditions. **Polyclonal gammopathies** are characterized by a broad-based electrophoretic peak that is composed of a heterogeneous mixture of immunoglobulins (Figure 1). This increased protein fraction is usually found within the γ-globulin region, but may extend into the β-2 region. Accompanying increases in acute-phase protein production, characterized by increases in α or β fractions, may also be noted. Polyclonal gammopathies can be noted with many causes of chronic tissue inflammation or infection, including chronic pyoderma, pyometra, and feline infectious peritonitis.

Monoclonal gammopathies typically occur as a single, narrow based, electrophoretic peak anywhere within the globulin region (Figure 2). Monoclonal peaks are due to the production of a homogeneous immunoglobulin molecule by a single clone of B lymphocytes or plasma cells. Causes of monoclonal gammopathies include lymphoma, lymphocytic leukemia, multiple myeloma, and plasmacytoma. Infrequently, monoclonal gammopathy may accompany disorders such as ehrlichiosis and canine amyloidosis.

Protein electrophoresis is offered weekly within the Clinical Pathology Laboratory, AHL at a cost of $40.00 per sample. 0.5 mL of serum is required. *AHL*

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AHL Newsletter

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Its mission is to inform AHL clients and partners about AHL current activities, and laboratory-based animal disease events and disease trends.

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

Ingestion of Russian knapweed causes nigropallidal encephalomalacia in horses, resembling Parkinson's disease

Margaret Stalker, Melanie Philbin, Brent Hoff

Russian knapweed (*Acroptilon repens*, syn. *Centaurea repens*), a perennial member of the Aster family, is native to Eurasia. Introduced into North America in the early 1900s, it is classified as a noxious weed in Ontario and elsewhere. The plant has erect stems up to a meter tall with simple alternate leaves covered in fine hairs, and rounded pink to purple flower heads produced singly on the ends of branches subtended by small pearly bracts with papery margins (Fig 1), flowering from July to September. Highly adaptable to many soil types, Russian knapweed can form dense stands in disturbed areas including roadsides, pastures and cropland, spreading by deep rhizomes, out-competing other species and reducing crop production by allelopathic effects.

The putative neurotoxin produced by Russian knapweed is the guaianolide sesquiterpene lactone, **repin**, which can cause glutathione depletion and oxidative neuronal damage. Intoxication by ingestion of Russian knapweed occurs only in horses, producing a syndrome of neurologic disease resembling Parkinson's disease in humans, with specific targeting of damage to the basal ganglia of the brain (globus pallidus and substantia nigra - nigropallidal encephalomalacia). Horses grazing large quantities of the plant for prolonged periods of time, particularly during dry summers when other forage may be limited, may develop abrupt onset of clinical signs characterized by impaired eating and drinking, general apprehension followed by pronounced depression with hypertonicity of the lips and tongue. This increased muscle tone may produce a fixed facial expression and rhythmic chewing or writhing movements of the tongue and lips, giving rise to the common name, "chewing disease". Death can result due to dehydration and starvation.

While disease has not been reported in Ontario within recent years, Russian knapweed is widespread in southern and central Ontario, and its poisonous potential should be recognized. (Plant specimen in the McNab Teaching Garden courtesy of Dr. Beverley McEwen, from a local pasture). *AHL*

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http://www.invasiveplants.ab.ca/Downloads/Acroptilon_repens.pdf

Canadian Poisonous Plants Information System: http://www.cbif.gc.ca/pls/pp/ppack.info?p_psn=221&p_type=all&p_sci=comm&p_x=pp

Figure 1. Russian knapweed (*Acroptilon repens)*, closeup of inflorescence. Photograph (c) *Barry A. Rice* / The Nature Conservancy http://tncweeds.ucdavis.edu/esadocs/acrorepe.html

Hyperproteinemia - continued from p. 26

Figure 1. Polyclonal gammopathy

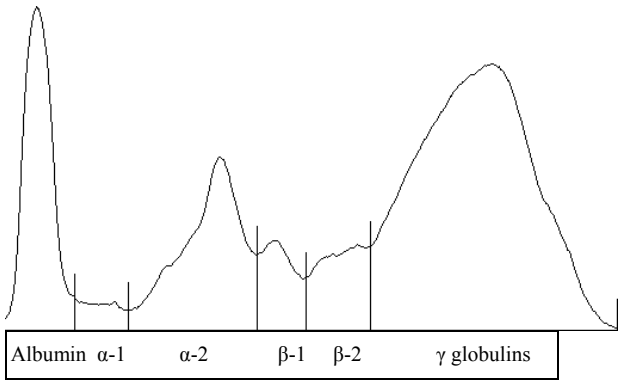
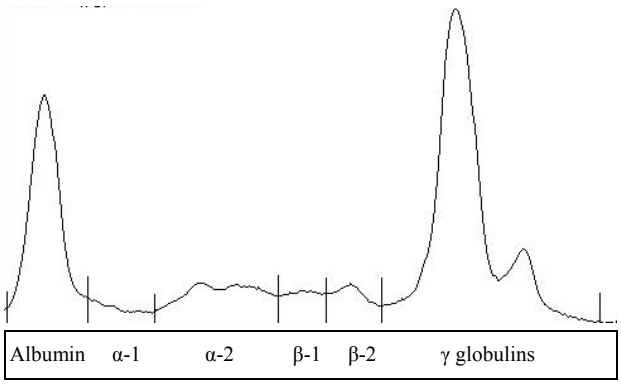


Figure 2. Monoclonal gammopathy



AHL Lab Reports

RUMINANTS

Myositis caused by *Clostridium chauvoei* in cattle

Murray Hazlett, Jan Shapiro, Brian Binnington, Durda Slavic

Blackleg (*Clostridium chauvoei* myositis) is characterized by dark areas of hemorrhage with emphysema in skeletal and/or heart muscle (Table 1). Affected muscle often has a dry appearance, and may smell like rancid butter. Other lesions may include fibrin on the pleura or epicardium, hemorrhages elsewhere in the carcass, and a predisposition for the carcass to autolyse quickly. It is not uncommon to see blood dripping from the nose, and often more than one animal is affected. Clinicians and pathologists sometimes also consider anthrax when examining these animals, unless they find the characteristic muscle lesions. Blackleg cases don't have the severe splenic congestion seen in anthrax cases.

Muscle lesions can be difficult to locate, and may involve turning the animal over, and/or skinning the entire carcass. In the 26 cases reviewed, 21 (81%) involved skeletal muscle, 11 (42%) heart, and 12 (46%) pleura/ pericardium. When specified, skeletal muscle lesions were most frequent in hip and back leg (n=6), back (longissimus dorsi region, n=6) and neck and shoulder (n=4). Three cases had severe acute transmural necrohemorrhagic inflammation of the abomasum, small intestines, or mesentery and mesocolon, as well as multiple discrete dark dry areas of myocardial necrosis. Histology was performed on 2 of these latter cases, and non-skeletal muscle tissues had the same lesions and intralesional bacteria as seen in affected skeletal muscle. One unusual case occurred in housed dairy cows that had not been on pasture for several months prior to illness. Case records indicated an average of 3 cattle affected on each premises at the time of submission.

Lesions are most easily identified in recently dead animals, and diagnosis is confirmed by histology and/or fluorescent antibody testing. In addition to *C. chauvoei*, *C. novyi*, *C. septicum* and *C. sordellii* are also included in routine FA testing, and are also sometimes present.

Most submissions to the AHL are in the late third or early fourth quarter of the year (Figure 1), perhaps because of moisture or pasture conditions that allow for proliferation of the bacteria in the environment. The disease is seen fairly evenly province wide. **We have seen no cases of the disease in vaccinated animals.** *AHL*

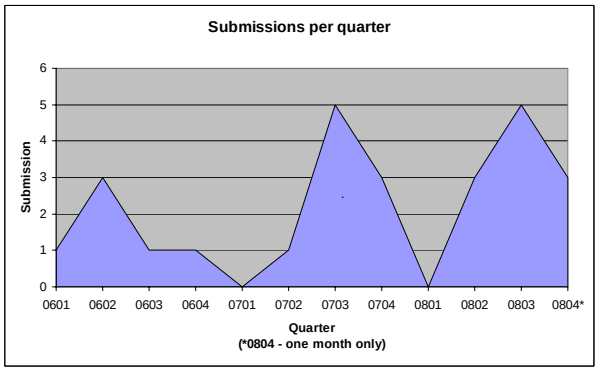


Figure 1. Blackleg cases diagnosed by the AHL, per quarter, 2006-2008.

Arthrogryposis multiplex - a new birth deformity in Angus calves

Ann Godkin

A deformity in newborn black Angus cattle is now recognized. Originally named "curly calf syndrome", arthrogryposis multiplex (AM) is now the preferred term. **Affected calves are born dead or die shortly after birth, are small, have bent or twisted spines, and rigid, hyperextended or contracted limbs**.

The history of the investigation of AM, and the ongoing work of Dr. David Steffen at Nebraska and Dr. Jon Beever at Illinois, can be found on the website of the American Angus Association at www.angus.org .

The cause is suspected to be an autosomal recessive trait. Carriers have been identified among Angus bulls from 5 insemination centers. The list is on the American website.

No cases have been reported to date in Canada, however the ancestry of sires and cows present in Canada can be traced back to a common ancestor, a bull named "Precision 1680".

Suspected cases should be reported to the Canadian Angus Association using the Calf Abnormality Report (available from www.cdnangus.ca) or by calling 1-888-571-3580. Photographs should also be taken and submitted with the form to Camille Scott (camille1@cdnangus.ca). Further direction on collecting tissue and/or blood samples will be provided upon receipt of the abnormality report. *AHL*

Abortions due to IBRV in a dairy herd

Murray Hazlett, Lance Males, Tony van Dreumel, Susy Carman

In early September, serum, tissue samples, and 2 fetuses were submitted to the AHL for abortion workups. The herd of 70 cows had experienced 6 abortions over 7 days. Except for one 3-year-old, all of the animals that aborted were 2-year-olds. Abortions occurred in the late second to early third trimesters. These cows did not retain placentas and came back into heat normally. Most of the cows had been vaccinated, albeit over a year previously, with a live intramuscular vaccine for *Bovid herpesvirus 1* (BHV-1, Infectious bovine rhinotracheitis virus, IBRV) - those that aborted had never been vaccinated. Three 1-week-old heifers had been recently purchased from a sales barn but kept isolated. Milk production was normal, and there had been no noticeable increase in respiratory disease.

Gross lesions were minimal - there was moderate autolysis with placental edema, cupping of cotyledons, and increased opacity of intercotyledonary zones. **Histology showed classic IBR lesions** - variable multifocal necrosis in liver, lung and spleen. There was mild placentitis. Immunohistochemical staining for IBRV antigen was done on 1 calf (liver (Figure 1), kidney, spleen, thymus) to confirm the diagnosis, and all showed strong staining except spleen.

Subsequently, IBR virus was isolated from one of the fetuses (pool of lung, liver, kidney, spleen, thymus and thyroid), but not the one with the positive IHC, **suggesting that both IHC and virus isolation are important for identifying IBR virus in bovine abortions.**

Of a total of 709 bovine pathology abortion submissions examined at the AHL since 1998, there were 37 cases of IBRV abortion (5.2%). *AHL*

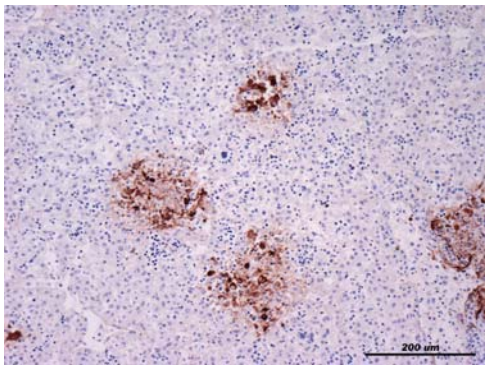


Figure 1: Immunohistochemistry demonstrating IBRV antigen staining in necrotic foci in liver.

Hemonchosis in small ruminants

Maria Spinato, Janet Shapiro, Brian Binnington, Murray Hazlett, Andrew Peregrine

The AHL reported an increased number of necropsy submissions related to hemonchosis in sheep and goats during the summer of 2008. Twenty-four cases, some with several affected animals, were diagnosed this year compared to only 8 cases in 2007. Clinical signs reported by producers included weakness, weight loss, diarrhea and sudden death. Gross findings at necropsy typically consisted of emaciation, severe anemia, peritoneal and pleural effusion, and subcutaneous edema that was especially prominent in the intermandibular region ("bottle jaw"). Masses of thread-like "barber-pole" nematodes approximately 1.5 cm in length were present within the red-tinged to melenic fluid content of the abomasum, consistent with *Haemonchus contortus*.

There are several factors implicated in the increased losses due to gastrointestinal parasitism this year. The wet summer weather in Ontario provided ideal conditions for prolonged survival of L3 larvae on pasture, compared to the much drier conditions in 2007, and likely was the major contributing factor. Additionally, returning de-wormed sheep to heavily contaminated pastures was seen as a significant factor in re-infection and prolongation of the disease in flocks. However, **multidrug-anthelmintic resistance is also an increasing problem**, reported globally as a cause of significant morbidity and mortality, occasionally resulting in flock culls. Resistance of *Haemonchus sp*. to both ivermectin and albendazole has recently been described on a farm in Ontario, and anecdotally, such resistance appears to be a problem on many farms. Animals from 16 of the affected farms in Ontario this year had been de-wormed with ivermectin or a benzimidazole product, and 3 producers had used both classes of anthelmintics to treat their flocks. Levamisole is a third drug that can be used in cases of suspected anthelmintic resistance. However, it must be compounded by request, as a commercial product is not available in Canada.

Two new classes of anthelmintics offer some hope that additional products will soon be available to manage drug-resistant nematodes: the cyclodepsipeptides, currently formulated for use only in cats, and the amino-acetonitrile derivatives, still in discovery phase. Until these new drugs are licensed for use in small ruminants, **control measures combining pasture management and an effective de-worming program are critical in preventing death losses due to hemonchosis**. **Practitioners and producers are advised to monitor for the efficacy of anthelmintic therapy by performing a fecal egg count reduction test each year in July/August**, as described in the September 2006 AHL Newsletter. *AHL*

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Diagnostic approach to neonatal calf diarrhea

Durda Slavic, Josepha DeLay, Susy Carman, Hugh Cai

Neonatal calf diarrhea (NCD) is a multifactorial clinical condition frequently affecting calves up to 30 days of age. Infectious agents most frequently associated with this condition include enterotoxigenic *Escherichia coli* (ETEC) *Rotavirus, Bovine coronavirus* and *Cryptosporidium parvum*. Numerous other predisposing factors can contribute to the onset of diarrhea, including failure of antibody transfer, poor nutrition and environmental and management conditions. The main focus of this article is the investigation of potential infectious causes of NCD through the efficient and economical use of diagnostic tests (Table 1).

The quality of diagnostic results is directly related to the quality of the specimens submitted to the laboratory. **The best diagnostic specimen from NCD case is feces collected in a clean container**. This fecal sample can then be used for a multitude of testing. Note that fecal swabs can be used only for bacterial culture. Ideally, samples for bacteriology should be collected prior to starting antimicrobial therapy.

When requesting diagnostic tests for NCD, **the age of the animal affected can be an indicator of infectious agents present**. Equally important, etiologic agents will often not be identified unless specific tests are requested. In NCD submissions to the AHL in 2003, multiple pathogens were found in 22% of cases, highlighting the importance of requesting the tests that will detect these multiple pathogens.

Table 1. Infectious agents most frequently associated with neonatal calf diarrhea, and tests offered by AHL for their detection

Age (days)	Infectious agent	Sample for testing	Tests recommended	Additional tests available/Comments
0-5	Enterotoxigenic *E. coli* (ETEC)	Feces or fecal swab	Culture and susceptibility **NOTE:** animal age must be specified in order to test *E. coli* isolates for the presence of F5	ETEC PCR (strongly recommended if slide agglutination was negative for F5 up to 2 days of age)
1-15	*Rotavirus A*	Feces, intestinal content, jejunum, ileum, colon	*Rotavirus group A* latex agglutination (RLA)	Protection depends on milk antibody. Adult carriers occur, with stress-induced shedding at parturition. Electron microscopy is required to identify the less common group B and C strains.
7-30	*Bovine coronavirus*	Feces, intestinal content, jejunum, ileum, colon	*Bovine coronavirus* antigen detection ELISA	Protection depends on milk antibody. Adult carriers occur, with stress-induced shedding at the time of parturition.
8-16	*Cryptosporidium spp*.	Feces from untreated calves in early diarrheic stages	Sucrose wet mount	

Escherichia coli is the main diagnostic concern in NCD cases involving very young animals (up to 3 days of age). However, not all *E. coli* present in feces are capable of producing NCD. ETEC is by far the most common type of *E. coli* capable of producing diarrhea. **ETEC isolates are characterized by production of fimbriae and toxin(s).** Fimbriae [mainly F5 (formerly K99)] allow bacteria to attach to the surface of enterocytes. Once attached, they start producing toxin(s) (primarily STa) which causes excess water secretion into the gut resulting in diarrhea. Morphologically, ETEC isolates do not differ from normal flora (non-disease producing) *E. coli* isolates. Therefore additional testing is required for their differentiation. The slide agglutination test for the presence of F5 is automatically done at AHL on *E. coli* isolates from NCD in animals up to 7 days of age. The specificity of that test is more than 99%, but sensitivity could be an issue. F5 fimbriae are not produced in vitro on the medium that is regularly used for bacterial isolation, therefore their production is stimulated by growth on special media. On rare occasions, F5 fimbriae are not expressed despite optimal conditions, resulting in a false-negative slide agglutination test. **If an F5 slide agglutination test is negative in animals up to 2 days of age and no other pathogens are isolated, then PCR testing for ETEC is highly recommended** (must be requested separately) - both fimbriae and toxin genes will be detected by this approach, confirming the presence of ETEC. We keep bacteriology plates for up to 3 days after results are released. If PCR is desired, please notify the laboratory promptly to ensure that isolates will be available for testing. For cases where autogenous bacterins are considered, PCR testing should be a must.

Rotavirus, Bovine coronavirus, and *Cryptosporidium parvum* are most common at 7-14 days of age (Table 1).

Necropsy in complicated cases allows sampling from a variety of tissues and pursuit of additional tests such as histopathology, virus isolation, and immunohistochemistry, which may identify less common causes of NCD. **Cases in which calves were submitted for post-mortem were more than twice as likely to obtain an etiologic diagnosis compared to cases where samples were chosen in the field.** Sample quality is important in this situation, as calves that have been treated or died may present diagnostic challenges due to antibiotic influence on bacterial culture results and autolysis of intestinal mucosa after death. **The most diagnostically useful samples are those from a recently affected, untreated calf whose tissues are harvested immediately following euthanasia**. For field cases**,** freezing several sections of small and large intestine and fixing tissues in formalin (preferably immediately following euthanasia) could increase etiologic diagnoses. *AHL*

AVIAN/FUR/EXOTIC SPECIES

Seroprevalence of *Turkey coronavirus* in Ontario and Arkansas

Maged Gomaa, Dongwan Yoo, Davor Ojkic, John Barta (In press, *Clinical and Vaccine Immunology*, 2008)

Turkey coronavirus (TCoV) causes diarrhea in young turkey poults, but little is known about its prevalence in the field. To address this, the nucleocapsid (N) gene of TCoV was cloned and expressed in *Escherichia coli*. The recombinant N protein was purified and used as antigen to develop an ELISA for serological detection of TCoV that was then validated using experimentally derived turkey serum. The N-based ELISA showed high sensitivity (97%) and specificity (93%) for TCoV, which was significantly higher than an *Infectious bronchitis virus* (IBV)-based commercial test for TCoV. To assess the utility of this recombinant ELISA, 360 serum samples from turkey farms in Ontario and 81 serum samples from Arkansas were tested for TCoV-specific antibodies. **High seroprevalence of TCoV was found on the Ontario farms with 73.9% of breeders and 60.0% of meat turkeys testing seropositive using the N-based ELISA.** Similarly high field prevalence was found in Arkansas where 64.2% of the serum samples tested seropositive. *AHL*

SWINE

Mycoplasma hyorhinis arthritis in organically raised pigs

Tony van Dreumel, Kevin Vilaca, Pat McRaild, Hugh Cai, Josepha DeLay

Mycoplasma hyorhinis is a common inhabitant of the respiratory tract in pigs, and infection may be associated with pneumonia and polyserositis. In post-weaning pigs, infection may be associated with arthritis only, without involvement of other serous membranes.

An outbreak of polyarthritis occurred in a 300-sow organic farrow-to-finish operation with negative herd status for PRRSV, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae*. The predominant clinical signs were lameness with swollen hock and carpal joints in piglets ranging from the early to late nursing stage. Up to 10% of pigs in this age group were at times affected.

All 6 untreated pigs submitted were in good body condition but had external evidence of swollen joints (joints were also submitted from 4 other affected pigs). Affected joints contained increased amounts of blood-tinged to cloudy fluid, and some contained fibrin. Synovial membranes were often edematous and pale red, and articular surfaces were eroded in one affected joint. There was no evidence of polyserositis in any of the affected pigs.

Microscopically, the joint cavities contained fibrinocellular exudate (Figure 1). The synovial membranes were hypertrophic and eroded in some areas. The stroma was edematous and had a marked, mainly plasmacytic, inflammatory reaction. The joint capsules were markedly thickened due to the presence of a mixture of inflammatory cells and fibrin. *M. hyorhinis* was isolated from either joint fluid or joint capsule from 4 of the 10 pigs cultured. No bacterial pathogens were cultured from any of the affected joints.

***M. hyorhinis* should be considered as a possible cause of arthritis in nursing pigs. Joint fluid and/or joint capsule are the specimens of choice for culture.** *AHL*

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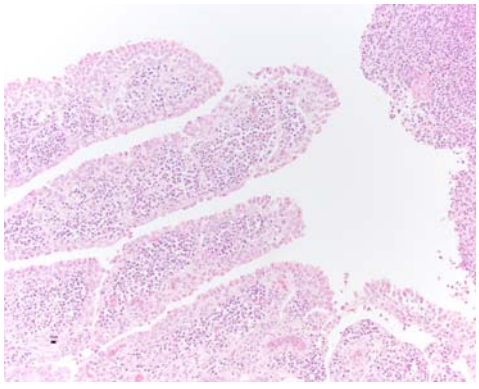

Figure 1: *M. hyorhinis* arthritis in a nursing pig. Fibrinocellular exudate in the joint cavity and plasmacytic reaction in the hypertrophic synovial villi.

Meningeal T-cell-rich B-cell lymphoma in a pig

Beverly McEwen, Josepha DeLay

A recumbent 74-kg market hog was submitted to the AHL for necropsy. Gross lesions included serofibrinous peritonitis, pericarditis and meningeal opacity, all suggestive of *Streptococcus suis* infection. Histologically, the meninges were diffusely and markedly infiltrated by closely packed round cells with round to occasionally indented or polygonal nuclei, usually containing a single prominent nucleolus, and scant eosinophilic cytoplasm. There was marked anisokaryosis/anisocytosis, and up to 5 mitotic figures per HPF. These neoplastic cells percolated into the underlying neuropil, surrounding cerebral and cerebellar vessels and were present beneath the ventricular ependyma. The neoplasm was restricted to the brain and meninges.

The immunohistochemical (IHC) staining pattern of infiltrating cells in the CNS was consistent with T cell-rich B-cell lymphoma (TCRBCL). Over 80% of pleomorphic round cells in meninges stained with antibody to CD79a, indicative of B-lymphocyte origin and, in the context of the cytologic features of the cell population, consistent with a diagnosis of B-cell lymphoma. Scattered clusters of smaller round cells stained with antibody to CD3, reflecting infiltrating (reactive) T-lymphocytes. The majority of round cells also stained with antibody to MHC II, a marker of activated antigen-presenting cells. IHC for PCV-2 was negative. *S. suis* was isolated from the spleen, but not the brain, of this pig.

Lymphoma is the most common tumor identified in swine at the AHL, and neoplasia is suspected in about 50% of these cases by the referring veterinarian. Some pigs are submitted as part of a group with various clinical signs, including respiratory signs, diarrhea, lameness or sudden death. IHC typing of lymphoma in pigs is not done routinely, however, the unusual infiltrate restricted to the meninges and brain in this pig warranted further investigation.

Multicentric TCRBCL is a rare histological variant of B-cell lymphoma described in people, dogs, cats, horses, cattle and pigs. TCRBCL in animals is usually nodal, however, extranodal involvement occurs, particularly in horses and cats. To our knowledge, **this is the first report of TCRBCL restricted to the meninges and brain of a pig.**

AHL

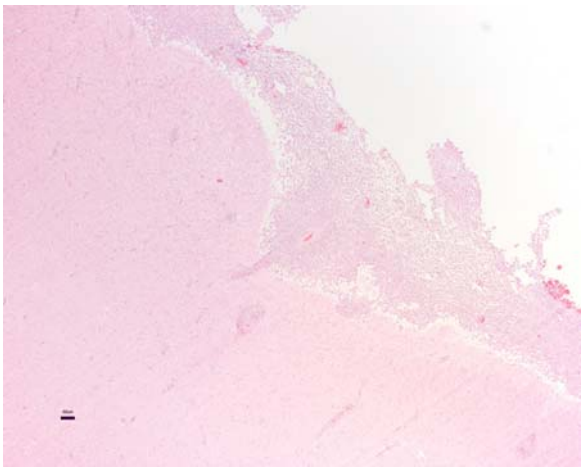

Figure 1. Expansion of meninges and formation of perivascular cuffs in underlying brain by neoplastic B lymphocytes, H & E stain.

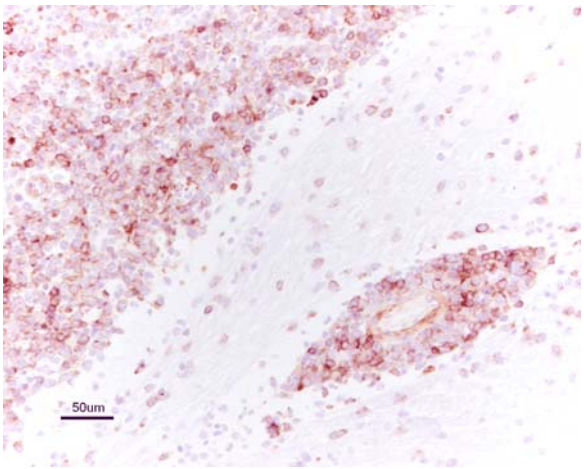

Figure 2. Immunohistochemical staining for CD79a in neoplastic lymphocytes in meninges.

LIMS-Sapphire - on-line access to results

To sign up for access to the client-only section of the AHL website and Web access your results, please contact:
Ms. Josie Dobing , Client Services (519) 824-4120, ext. 54320 jdobing@lsd.uoguelph.ca , or
Dr. Jim Fairles, AHL Client Services Veterinarian (519) 824-4120, ext. 54611
jfairles@lsd.uoguelph.ca

Fewer PRRSV-positive cases identified in 2008

Susy Carman, Beverly McEwen, Jim Fairles

Although new PRRSV outbreaks are still occurring, only 14% (44/305) of all swine cases tested in July-Sept 2008 were identified as PRRSV PCR-positive (Figure 1), which is less than the 22% (75/338) tested positive in April-June 2008 and less than the 31% (120/392) that tested positive for Jan-Mar 2008. Although this decline in 2008 also represents seasonal variation, this is less than in previous July-Sept intervals with 20% (73/364) positive in 2007, 29% (103/353) in 2006, 39% (117/297) in 2005 and 51% (48/94) in 2004 at the beginning of the outbreak. The annual average percent positive tests have declined from 51% in 2004 to 45% in 2005, 33% in 2006, and 27% in 2007. These data do not include semen monitoring cases. However it was not possible to exclude serum or blood swab monitoring cases, such that testing may represent enhanced monitoring of swine herds with ongoing infections, rather than be all new outbreaks.

The AHL offers many diagnostic tests to **identify PRRSV** in swine herds including the Tetracore NA/EU real time RT-PCR, which identifies both North American (NA) and European-like (EU) strains in a single test, immunohistochemistry (IHC), histopathology, fluorescent antibody tests (FAT), and PRRSV gene sequencing to monitor virus strains. As well, the AHL offers a number of tests to **identify PRRSV antibody** including the IDEXX PRRSV 2XR antibody ELISA (with antibody identified by 9-11 days), and indirect fluorescent antibody (IFA) tests for both IgM (with antibody identified by 5 days) and IgG (with antibody identified by 7-11 days) for NA, EU (Lelystad) and NA Euro-like (South Dakota) strains of PRRSV.

Both the Tetracore NA/EU real time RT-PCR and the IDEXX PRRSV 2XR antibody ELISA are now performed daily Mon-Fri at the AHL and are used to monitor swine herds for new outbreaks of PRRSV. Where singleton positive PRRSV antibody ELISA tests occur in PRRSV-negative herds, we recommend testing these sera using both the real-time RT-PCR and IFA tests. Use of both IgM and IgG tests with different strains of the virus may identify specific antibody a few days earlier, but viremic animals should be identified during this time using PCR.

The AHL routinely tests sera in the NA IgG IFA, since all virus strains sequenced at the AHL have been NA strains. However, the herd veterinarian, who knows the source of replacement pigs and history of PRRSV sequencing in the herd, should request the additional types of IFA tests to be performed. The ELISA and the IFA tests are run the same day if the serum arrives in the lab by 9:00 AM. Label the outside of the box "**PRRSV testing**" so these shipping boxes are opened first. Samples that arrive later can be tested on the same day if needed, with an additional charge if out-of-hours testing is required. It is helpful when instructions are written on the submission form, "**please do IFA and PCR on all PRRSV antibody-positive ELISA**", and we will initiate this testing as soon as the ELISA is completed. Same-day testing following the ELISA is normal for the IFA. The AHL runs the Tetracore NA/EU real time RT-PCR daily Mon-Fri with results available the next business morning. Same-day PCR testing and emergency testing are available at additional charges. Please call the lab for further information. *AHL*

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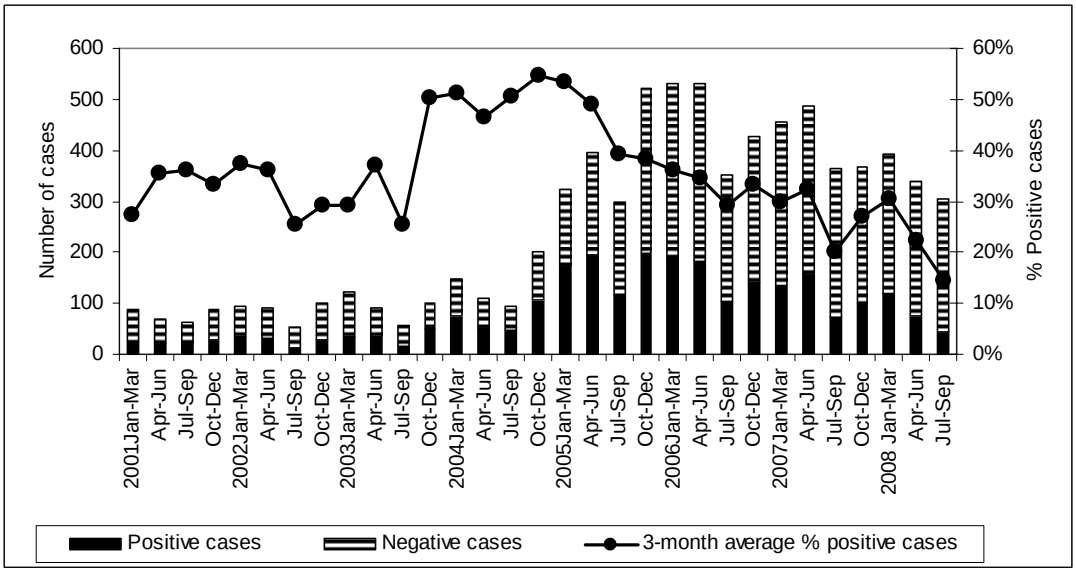

Figure 1. PRRSV-positive cases by PCR, 2001-2008, AHL.

HORSES

Equine viruses identified or isolated at the AHL, 1998 to October 2008

Susy Carman, Beverly McEwen, Josepha DeLay, Davor Ojkic

The AHL offers a full range of diagnostic tests to demonstrate equine viruses in clinical specimens, including virus isolation in cell culture and eggs, fluorescent antibody testing (FAT), antigen detection ELISA, polymerase chain reaction (PCR), immunohistochemistry (IHC), and IgM ELISA. Table 1 records the number of various viruses identified in horses from January 1998 to October 2008.

Equid herpesvirus 1 (EHV-1), or equine abortion virus, is the virus most regularly identified in horses in Ontario. EHV-1 is most commonly identified from aborted fetuses and is the most common viral agent causing abortion, with some EHV-1 associated with neonatal deaths, and a few being associated with neurological cases. Three cases of the neuropathic strain of EHV-1 have been demonstrated in horses since specific testing began in late 2007. ***Equid herpesvirus 4*** (EHV-4), or rhinopneumonitis virus, is uncommonly identified, and has only rarely been associated with abortion. ***Equid herpesvirus 2*** (EHV-2), or cytomegalovirus, can be identified in normal horses and is also associated with upper respiratory disease with lymphadenopathy in adults and pneumonia in foals.

Both ***Equine rhinitis A virus*** and ***Equine rhinitis B virus*** (previously termed *Equine rhinovirus 1* and *2*, respectively) continue to be associated with fever, upper respiratory disease, and ventral and limb edema. As in the past, all of the ***Influenza A virus*** identifications from outbreaks of respiratory disease in horses have been H3N8.

In 2002, outbreaks of both ***West Nile virus*** (WNV) and ***Eastern equine encephalitis virus*** (EEEV) were identified, with cases of WNV continuing until 2005. In the summer of 2008, another outbreak of EEEV occurred. Sporadic outbreaks of EEEV occurred in Ontario in 1992 and 1994. **Rabies** has been identified more commonly in recent years.

Although ***Equine arteritis virus*** is endemic in the Standardbred horse population in Ontario, this virus has only rarely been identified as a cause of abortion or clinical illness in horses in Ontario. *AHL*

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Table 1. Viruses identified or isolated from horses at the AHL, January 1998 – October 2008

Virus	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
EHV-1[1,3,5] (abortion virus)	3	14	12	10	12	13	22	9	12	8	11
EHV-4[1] (rhinopneumonitis virus)	1			1	1	1	1		1	1	1
EHV-2 (cytomegalovirus)[1]	6		1		1		1	3			
Equine rhinitis A virus (1)[1]					1		1				
Equine rhinitis B virus (2)[1]							3	1		1	
Influenza A virus[2,4,5,6] (H3N8)				1	1	5	3	1	1		1
West Nile virus[5,6,7]					84	3	10	4	0	0	3
Eastern equine encephalitis virus[5,6,7]					10	2					8
Picornavirus[1]					1						
Rabies virus[1,3]									1	2	1
Equine arteritis virus[1]			1								

[1]Virus isolation, [2]Egg inoculation, [3]FAT, [4]ELISA, [5]PCR, [6]IHC, [7]IgM ELISA

COMPANION ANIMALS

Anticoagulant rodenticide poisoning, what we can learn retrospectively

Brent Hoff, Jen Thompson, Judy Brown

A 3-year-old mixed-breed dog was referred to the Ontario Veterinary College (OVC) because of acute dyspnea. The previous day, the owners noticed that the dog was acutely lethargic and walking abnormally and phoned a local veterinarian for advice. **The owners were advised to induce emesis by force-feeding their pet salt; within hours of vomiting, the dog developed severe respiratory distress.** The dog was diagnosed at OVC with aspiration pneumonia, based on clinical signs, radiographs and cytology of bronchiolar lavage fluid. A complete blood count revealed an elevated hematocrit, mild leukocytosis attributable to neutrophilia, and a left shift. Biochemistry revealed mild hypoalbuminemia, elevated urea and mild electrolyte imbalances. A coagulation profile revealed elevated prothrombin time partial thromboplastin time, increased fibrinogen, however platelet levels were within normal limits. Despite aggressive therapy, which included 3 days of antibiotics, intravenous fluids and mechanical ventilation, the dog's condition deteriorated and he was euthanized.

This case underlines the importance of early detection and proper decontamination methods for cases of acute toxicosis

At necropsy, the lungs were diffusely dark red, heavy and firm on palpation; on cut surface the airways oozed copious amounts of dark-brown opaque fluid. Multiple superficial gastric ulcers were also present. Microscopically, there was severe fibrinonecrotic and suppurative bronchiolitis and alveolitis, centered on airways which contained abundant food material and fibrin; in more severe areas (left caudal and middle lung lobes), there was regionally diffuse hemorrhage. Based on the clinical presentation and coagulation tests, samples were submitted for anticoagulant testing and tissues were positive for bromadiolone (0.5ug/g).

Bromadiolone is a second generation anticoagulant used as a rodenticide and is found in brands such as Contrac, Maki, and Hawk. The second generation anticoagulants have increased single feeding lethality as the biologically active time frame is greater than for first generation classes, which often require multiple feedings to produce toxicosis. The oral LD_{50} of bromadiolone in dogs is reported as 0.5-15mg/kg and toxicity is seen with ingestion of greater than or equal; to 1/10 of the LD_{50}. The mechanism of action of bromadiolone is inhibition of epoxide reductase, which is needed to generate vitamin K; vitamin K dependent clotting factors (II, VII, IX, X) are consequently not formed and clinical signs are observed after circulating factors are depleted (3-7 days post-exposure). The compounds most commonly sold in Ontario include: warfarin, bromadialone, brodifacoum, pindone, chlorophacinone, and diphacinone.

There is a gradual onset of clinical signs that vary depending on the site where bleeding occurs - lameness (bleeding into joints), epistaxis, rectal bleeding and dyspnea are among typical presenting signs. Early detection of exposure is possible with coagulation assays, and prothrombin (PT) times will be prolonged prior to clinical signs. Prognosis depends on the dose, early diagnosis and early decontamination (emesis); treatment with vitamin K1 is highly effective.

In the case of this patient, the majority of the clinical signs were attributable to aspiration, rather than anticoagulant toxicity as exposure was very acute. This case underlines the importance of early detection and proper decontamination methods for cases of acute toxicosis. Similar cases have been documented in this laboratory. **The practice of instructing owners to perform at-home oral gavage with salt is potentially dangerous and should be strongly discouraged**. Safer and more reliable emetics such as 3% hydrogen peroxide and apomorphine are currently available and should be given within 1 hour of exposure. Emesis should never be induced in individuals once signs of poisoning are present. Emetics should not be given or repeated if vomiting has already occurred.

Analytical methods are available to identify the specific anticoagulant compound in order to institute treatment and establish the dose and length of vitamin K1 therapy. Exposure to anticoagulant rodenticides is most commonly confirmed by measurement of the specific compound in stomach content, source material, or liver samples. *AHL*

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Mycobacterium tuberculosis infection in a dog

Margaret Stalker, Durda Slavic

A 7- year-old neutered male Bichon Frisé dog was received for post-mortem examination at the Animal Health Laboratory. An abdominal mass had been diagnosed in the dog ~2 months prior, and the dog had recently become depressed, developed a low-grade fever, nasal discharge and pneumonia. An exploratory laparotomy revealed that the abdominal mass was an enlarged mesenteric lymph node, which was biopsied and submitted to a local diagnostic laboratory. The histologic diagnosis was granulomatous lymphadenitis with acid-fast bacilli, and preliminary testing indicated that the isolate was a member of the *Mycobacterium tuberculosis* complex (which includes *M. tuberculosis*, *M. bovis, M. africanum, M. microti* and *M. canetii*). The dog was subsequently euthanized due to the poor prognosis and risk of transmission to household members, including children and other pets.

The necropsy was performed in a biological safety cabinet using strict safety precautions to reduce the potential of exposure for laboratory personnel. The dog had remarkable enlargement of the cranial mediastinal and tracheobronchial lymph nodes (Figure 1). All lung lobes had irregular areas of pallor and congestion, and small 2-3 mm foci of consolidation. The omentum and intestinal loops adhered to the surface of a massively enlarged ileocecocolic lymph node (9 cm diameter), creamy white on cut section with central areas of caseation necrosis. On histology, granulomatous pneumonia, lymphadenitis and hepatitis with intralesional acid-fast bacilli were diagnosed. Samples of lung, ileocecal and bronchial lymph node and tonsil were sent to the Central Public Health Laboratory, Mycobacteriology lab, where *Mycobacterium tuberculosis* complex was identified by the amplified mycobacterium tuberculosis direct (AMTD) test and further confirmed by culture. The final speciation of *M. tuberculosis* complex was done using the GenoType Mycobacterium MTBC line-probe assay.

This case represents the first diagnosis of *M. tuberculosis* in the AHL's case files. Dogs may be infected by organisms from both the *M. tuberculosis* complex and the *M. avium* complex (which includes *M. avium, M. intracellulare* and *M. avium* subspecies *avium, silvaticum* and *paratuberculosis*), as well as other saprophytic opportunistic mycobacteria. While infection is often subclinical, dogs may develop pulmonary, gastrointestinal or disseminated disease, as in this case. As clinical signs associated with infection are not etiologically specific, rapid speciation of the infecting organism is desirable in order to ascertain the health risk to humans and other in-contact animals. In this case, preliminary results were available from our colleagues in Public Health within 3 days. The source of the infection for this dog is speculative.

Dogs are considered relatively resistant to *M. tuberculosis* infection, however there are several reports in the literature documenting transmission from human patients with active tuberculosis to in-contact dogs as a form of "reverse zoonosis" or anthropozoonosis, a situation that veterinarians should be aware of, although reciprocal transmission from dogs back to humans, and dog-to-dog transmission is considered very rare.

Tuberculosis is a major re-emerging disease in human populations, and consequently the incidence of canine infections mirror the epidemiology of infection in the human reservoir hosts. *AHL*

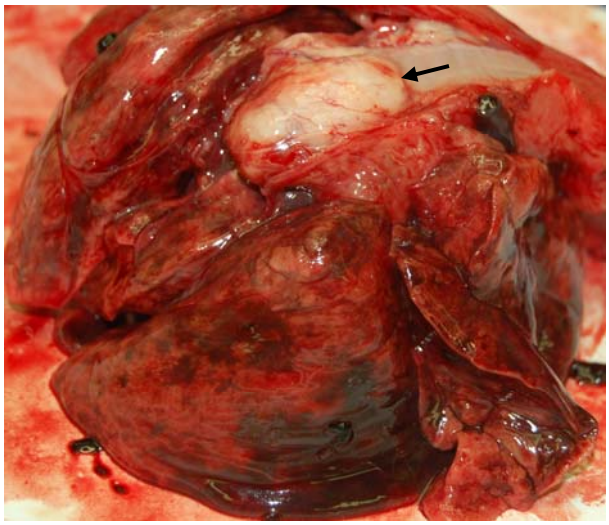

Figure 1: Massively enlarged tracheobronchial lymph node, granulomatous lymphadenitis due to *Mycobacterium tuberculosis* infection.

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