UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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Dr. Josepha DeLay, our newest ACVP diplomate!

We are delighted to announce that **Dr. Josepha DeLay, AHL Veterinary Pathologist,** passed the anatomic pathology examination of the American College of Veterinary Pathologists (ACVP) this fall, and is now officially a "**Diplomate ACVP**". Josepha joined the AHL in June 2000. A 1988 graduate of the Ontario Veterinary College, University of Guelph, she practiced in New Brunswick for 7 years before returning to Guelph and completing a DVSc in anatomic pathology at OVC in 1999.

Dr. DeLay provides pathology evaluation for a diverse range of food animal and companion animal species, and has been instrumental in the implementation of numerous new immunohistochemical techniques that extend our diagnostic capability.

Client satisfaction feedback

We mailed a feedback form with the September AHL Newsletter, and also visited several client clinics and hatcheries to get first-hand feedback on how we could improve service to you. Feedback and actions arising include:

- The AHL Newsletter, Fee Schedule, and User's Guide are well used and will continue to evolve to keep you informed.
- Fax is still the preferred way of reporting lab results, but email is also available.
- We are working on improved capture of client postal codes for tracking of provincial disease trends by OMAF through use of a separate postal code field.
- We will forward personalized waybills to clients on request. We accept incoming collect Purolator shipments from within Ontario if the University of Guelph account number **0966901** is quoted.
- Please note that payment options for your accounts include VISA, MasterCard, electronic bank transfer, debit, and money order, as well as traditional cheques.
- We will ensure that we enter barn/flock/farm information in client fields in order that results are matched with the correct site, and will work with clients to standardize these unique identifiers.

Proficiency panel success for Johne's culture!

Marie Archambault

The AHL is now an NVSL-approved laboratory for Johne's disease fecal culture, having successfully passed the National Veterinary Services Laboratory (NVSL), United States Department of Agriculture (USDA), Johne's (*Mycobacterium avium* subspecies *paratuberculosis*) fecal culture panel with our newly validated BACTEC test. This check test is performed annually by accredited laboratories and provides quality assurance to laboratories performing Johne's testing. Congratulations to our technicians **Jason Eidt** and **Muhammad Javid** on this success! For further information on our test validation, please refer to the Johne's article published in the September 2003 AHL Newsletter, p 27.

Specimens for diagnosis of enteritis

Peter Lusis, Marie Archambault, Susy Carman, Tony van Dreumel *plusis@lsd.uoguelph.ca*

A **complete history** (vaccination, treatment, age at onset, duration of clinical signs, etc.) is essential for selection of appropriate tests and interpretation of results.

- Intestinal autolysis and overgrowth of commensal bacteria occur very rapidly, and samples should be collected as soon as possible after death or within minutes of euthanasia.
- Live calves or piglets submitted as soon as possible after onset of diarrhea are recommended in herd outbreaks where a viral etiology is suspected.
- To avoid cross-contamination, tissues should not be pooled, and they should be submitted in separate labeled containers for each test.
- Please feel free to contact us for special enquiries or requests.

Bacteriology

Feces and/or fresh (chilled) samples of the lower small intestine, colon, and mesenteric lymph nodes, preferably from untreated animals, are recommended but frozen samples can also be submitted. Feces or colon contents are required for *Clostridium difficile* and *Clostridium perfringens* ELISA toxin detection assays. Please indicate the suspected etiology (*E. coli*, *Salmonella* spp., *Brachyspira* spp, *Lawsonia* spp., mycobacteria, *Campylobacter* spp., *Yersinia* spp., *Clostridium* spp., etc.) because special procedures are required for some tests.

Virology

Frozen sections of the small and large intestine, including Peyer's patches and any lesions, should be submitted for virus isolation. For BVDV, please submit lymphoid tissues (thymus, lymph node, spleen, Peyer's patches) and any tissues with lesions. Frozen feces are suitable for rotavirus and coronavirus tests. For fluorescent antibody tests, live piglets (TGEV, rotavirus) and calves (rotavirus) should be submitted.

Parasitology

Fresh or frozen feces are suitable for coccidia, cryptosporidia, gastrointestinal nematodes (except some *Strongyloides* spp.), cestodes, etc., but for some tests (*Giardia*, *Trichomonas*) fresh feces kept warm and submitted within hours of collection are required. Please indicate the suspected cause of diarrhea (cryptosporidia, gastrointestinal nematodes, *Strongyloides*, etc.) because procedures vary depending on tests required.

Histopathology

Three or four, 1-cm segments from each of the stomach, duodenum, jejunum, ileum (including Peyer's patches) and colon, and mesenteric lymph nodes, are usually adequate, but for some diseases (BVD, salmonellosis, and other systemic diseases) sections of the upper gastrointestinal tract and other tissues are also advisable. Immunohistochemistry (IHC) can also be done for detection of BVDV, TGEV, and bovine coronavirus (BCV).

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

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Show me some skin - obtaining useful information from skin biopsies

Josepha DeLay, Jan Hall, Sameh Youssef, Beverly McEwen, Natalie Keirstead, Joanne Dias
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Zack, an 11-year-old, intact male mixed-breed dog, was presented with a 2-month history of alopecia, crusting, and pruritus involving the nose, ear margins, trunk, and footpads.

- 2 biopsies taken from the skin over the trunk demonstrated severe epidermal hyperplasia with infiltration of eosinophils around blood vessels in the superficial dermis.
- The histologic lesions were **most suggestive of ectoparasite infestation**, with sarcoptic mange as the primary differential diagnosis.
- The dog did not respond to therapy for ectoparasites or with antibiotics, and was re-examined 2 months later, with identification of similar lesions that had extended to involve axillae.
- 11 additional biopsies were taken from all affected sites, including nose and ear margins, and samples from the face and ears contained intraepidermal eosinophilic pustules with acantholytic cells, **consistent with pemphigus foliaceus**.
- Biopsies from the trunk had lesions similar to those evident in the initial biopsies, and lacked the diagnostic acantholytic pustules.

This case illustrates several very important considerations when delving into the sometimes frustrating realm of dermatologic disease and skin biopsies:

1. Don't be cheap with skin biopsies - take multiple biopsies from multiple sites, including all (or at least several) affected areas. In Zack's case, the initial biopsies were not truly representative of the real disease process and actually acted as a 'red herring', leading the clinician and pathologist down an unsatisfying path. Multiple biopsies from several affected sites can lead to a more useful diagnosis; in Zack's case, he has responded well to therapy based on the final diagnosis of pemphigus foliaceus.

If lesions have a different clinical appearance in various sites on the body, place the biopsies in separate formalin jars labeled with each biopsy location. Many animals have multiple disease processes occurring concurrently in skin, and it is important to survey all affected areas in order to fully evaluate the lesions. Your pathologist can provide a more accurate dermatologic diagnosis from multiple biopsies, as this will in effect increase the sample area and will improve the chances of capturing diagnostic lesions. Taking multiple biopsies is even more important in large animals, such as horses, where the surface area of skin is that much greater. *A minimum of 6 skin biopsies is recommended to evaluate most equine and food animal dermatology cases.*

2. Always include lesion distribution in your dermatologic history. Most dermatologic diseases have specific anatomic distributions, and pathologists attempt to correlate the microscopic appearance of the biopsies with their location on the animal. In Zack's case, lesions involving the face, ears, and trunk, with eventual extension to axillae, were compatible with expected distribution of lesions in sarcoptic mange; footpad involvement was atypical for this disease, but biopsies were not examined from this location and could have represented a separate disease entity. The final diagnosis of pemphigus foliaceus is more compatible with this lesion distribution, including involvement of the footpads. Although pathologists prefer to initially read the histopathology without recourse to the clinical history, digital and photographic images may be helpful in providing the most complete description of the clinical problem.

3. Biopsy at an appropriate time in the course of disease – don't be too early or too late. Many clinical skin diseases are complicated by secondary, superimposed pyoderma. The histological picture can be clouded by lesions of pyoderma, and the 'real' lesions become much clearer when bacterial involvement is eliminated. As a result, some cases will benefit from an initial course of appropriate antimicrobial therapy prior to re-evaluation and biopsy. At the opposite end of the spectrum, changes induced in skin as a result of long-term inflammation due to any cause can completely mask the original insult, leading to non-specific diagnoses that are not helpful to the patient or clinician.

4. Glucocorticoids will influence microscopic lesions in skin and therefore affect the diagnosis. The effects of glucocorticoid therapy persist for a substantial period of time, and clinicians should allow 4 to 6 weeks between the last treatment and skin biopsy. The duration of therapy and date of last treatment should be included with the history, as this will affect the pathologist's interpretation of lesions.

5. If the histopathologic diagnosis does not fit with your clinical impression, or if your patient is not responding to therapy appropriate for that diagnosis, talk to your pathologist. A 3 or 6 mm punch biopsy cannot substitute for examination of the entire animal, and pathologists make the most useful diagnoses when microscopic lesions can be integrated into the clinical picture. Including your list of differential diagnoses in your history is an effective way of conveying your clinical impressions.

AHL Lab Reports

CATTLE

How to submit samples for Johne's fecal culture and direct fecal PCR

Marie Archambault, Jason Eidt, Muhammad Javid *marchamb@lsd.uoguelph.ca*

The AHL Bacteriology laboratory now offers 2 microorganism-based tests to detect *Mycobacterium avium* subspecies *paratuberculosis* (MAP):

- BACTEC fecal culture
- direct fecal PCR

The BACTEC culture is a radioisotope-based liquid culture method that requires the growth of the microorganism. The advantage of this method is that it can detect low numbers of MAP faster than traditional culture (8 weeks instead of 16 weeks). It can also grow MAP from a variety of species, including sheep. Negative fecal samples are incubated for a maximum of 8 weeks if submitted fresh and 12 weeks if previously frozen. The turnaround time for the BACTEC culture depends on whether the animal is a low, medium or high shedder - a fecal sample from a heavy shedder could take as little as 1 week to come up positive.

The PCR is performed directly on the feces submitted, and is therefore much faster than culture. The tentative turnaround time for the direct fecal PCR is 3 to 5 working days. However, according to our results to date, this test appears to be less sensitive than BACTEC culture.

Please forward 5 g of fresh feces for each test, indicate the date the sample was taken, and specify whether the fecal sample was frozen or not. If you are submitting a fresh fecal sample for BACTEC culture, we recommend that you send the sample on a Monday or Tuesday, as we start the culture process on Wednesday each week. If your sample has not been frozen, the turnaround time will be faster .

BACTEC culture is $25/fecal sample, and direct PCR is $20/fecal sample.

Please feel free to contact us if you have any questions.

Blackleg (*Clostridium chauvoei*) pericarditis

Peter Lusis, Marie Archambault, Beverly McEwen, Jan Shapiro *plusis@lsd.uoguelph.ca*

From August to mid-October 2003, the AHL diagnosed blackleg in 10 herds. Nine of these herds were from eastern Ontario, and where a history was available, none were vaccinated and all deaths occurred in pastured beef calves under 8 months of age.

In one herd, 11 of 60, 200-250 kg calves died suddenly over approximately 9 days, after 1-2 days of wet stormy weather. Most calves were found dead but one was hypothermic shortly before death. Cows on the same pasture were not affected.

Two calves were necropsied by the attending veterinarian and 2 were necropsied at the AHL. In all calves, there was **severe acute fibrinous pericarditis,** and in one calf there was acute necrosis of the right atrium of the heart. Gross or microscopic lesions were not seen in skeletal muscles of the 2 calves necropsied at AHL, but *C. chauvoei* was identified by fluorescent antibody (FA) testing of skeletal muscles and hearts of both calves. No other pathogens were isolated on routine bacteriology.

Although skeletal muscle lesions are present in most cases of blackleg, they may be very localized and not evident on routine gross or microscopic examination. However, **there is often focal or diffuse fibrinohemorrhagic pericarditis and pleuritis and, when these lesions are present without severe pneumonia, blackleg should be suspected,** even if lesions in skeletal or cardiac muscles are not evident.

Unlike *C. septicum* and other clostridia, *C. chauvoei* does not proliferate readily after death, and **smears from muscle or other lesions for *C. chauvoei* FA can be submitted even from partially autolysed tissues.**

Reference

Jubb KVF, Kennedy PC, Palmer N. Pathology of Domestic Animals. 1993; vol 1: pp 248, 249.

What is multidrug-resistant (MDR) *Salmonella* Newport?

Marie Archambault, Anne Muckle, Kim Klotins *marchamb@lsd.uoguelph.ca*

There has been an **increasing prevalence** of multidrug-resistant (MDR) *Salmonella enterica* serotype Newport infections, primarily in dairy cattle in Ontario (see AHL Newsletter, Sept 2003, pp 29-30). All *Salmonella* serotypes have the potential to cause infections in all animal species. Some *Salmonella* spp are susceptible to many antimicrobial agents, others have genetic elements that confer resistance to antibiotics. Some *Salmonella* Newport isolates display resistance to multiple antimicrobials via genetic elements called plasmids. These plasmids seem to be very effective in spreading resistance and acquiring new resistance genes. Recently, a significant proportion of Ontario isolates have demonstrated resistance to over 9 antimicrobials including ampicillin, amoxicillin/clavulanic acid, cephalothin, ceftiofur, ceftriaxone, cefoxitin, florfenicol, kanamycin, neomycin, streptomycin, sulfisoxazole, and tetracycline, while being susceptible to amikacin, apramycin, ciprofloxacin, gentamicin, nitrofurantoin, spectinomycin, sulfamethoxazole/trimethoprim, tobramycin, and trimethoprim.

Public health concerns

This emerging MDR pathogen was first documented from human cases in the United States in 1999, and in 2000 in Canada. **Human cases have been reported in Ontario. This organism is of public health concern since human infections have been linked to direct contact with infected dairy cattle, consumption of raw milk and products made from raw milk, and consumption of ground beef.** Salmonella infections in humans usually cause digestive tract infections and are self-limiting. On occasion, infections may become systemic and require antimicrobial therapy. MDR *Salmonella* Newport complicates treatment course due to its multidrug resistance.

Monitoring of resistance

Resistance of *S*. Newport isolates from both animals and humans is being monitored by Health Canada as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Recent human and dairy cattle infections indicate that this MDR serotype may be emerging here.

How to control MDR *Salmonella* Newport

To signal suspected cases, you can contact a veterinarian from OMAF Veterinary Science or the AHL. You can also submit fecal samples to the AHL for bacterial culture and antimicrobial susceptibility testing. Expanded bacteriology culture may help in understanding the distribution of the pathogen within the herd. **Strict biosecurity procedures should be initiated.** Farm family members should be advised that this pathogen could cause disease in humans and that they should protect themselves by adhering to correct personal hygiene protocols. Raw milk should not be consumed. Any individuals exhibiting signs of enteric disease should be directed to contact their family physician immediately. Information on *S*. Newport should be provided to the family physician.

The 4-page USDA bulletin "What veterinarians and producers should know about multi-drug resistant *Salmonella* Newport" contains valuable information, and is found at the following website: http:www.aphis.usda.gov/vs/ceah/cahm/What's New/Snewport.PDF

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Gupta A, et al. Multistate investigation of multidrug-resistant Salmonella serotype Newport infections in the Northeastern United States, 2000: Human infections associated with Dairy farms. 2000. Available at: http://www.cdc.gov/narms/pub/presentations/idsa/2001/gupta_a1.htm

Possible endotoxin-related vaccine reactions

Peter Lusis *plusis@lsd.uoguelph.ca*

Over the past few months, we have seen 6 cases of sudden death in dairy heifers and cows within several hours of administration of vaccines containing gram-negative bacterial components. In most cases, only one of several vaccinated animals was affected. Gross and microscopic pathology revealed acute pulmonary congestion and edema with no other significant lesions. We suspect that at least some of these deaths may be endotoxin-related.

Additive effects of endogenous endotoxins from subclinical infections, bacterial contamination of multi-dose vaccines, freezing of vaccines containing gram-negative bacteria, and/or administration of multiple vaccines within a short time of each other have also been suggested as causes of endotoxin-related vaccine reactions. According to one report, "Vaccines composed of gram-negative bacteria contain endotoxin in considerable amounts. This may result in adverse effects after vaccination of sensitive animals."

These and all suspected adverse vaccine reactions should be reported to:

Veterinary Biologics Section
Canadian Food Inspection Agency
Phone: 613-225-2342 Ext 4656; Fax: 613-228-6612

Reference

Cussler K, Godau H, Gyra H. Investigation of the endotoxin content of veterinary vaccines. ALTEX 1994;11:24-29.

POULTRY

Emus die from eastern equine encephalitis virus (EEEV) infection

Emily Martin, Davor Ojkic, Marie Archambault *emartin@lsd.uoguelph.ca*

For the first time, a case of eastern equine encephalitis in emus has been diagnosed at the AHL. Two dead adult emus were presented with a short clinical course (maximum 48 hr) of depression and recumbency. At the time of presentation, 3 emus had died on this farm, the birds had bloodstained vents, and anticoagulant toxicity was suspected; 1 emu had been treated with vitamin K and seemed to be doing better. On gross post mortem examination, there was marked accumulation of blood in the mouth and esophagus, the proventriculus and ventriculus had thick black material over the mucosal surface, the koilin layer of the ventriculus was black, and there was marked accumulation of serosanguinous fluid throughout the small intestines. There were multiple petechial to suffusive hemorrhages over the serosal surface of the small intestines. Other lesions included pericardial petechial hemorrhages, red-black mottling of the liver, enlarged red-black spleens, and multiple hemorrhages around the ovaries.

In view of the history and gross post mortem findings, special stains (polychrome methylene blue, M'Fadyean's reaction) were performed, but were negative for anthrax. Histologically, there was marked hemorrhage in the liver and mild hepatocellular necrosis, fibrinoid degeneration of sheathed arterioles in the spleen (Figure 1), and marked hemorrhage dissecting the muscularis layers of the small intestine. Brain, spleen and intestinal tissues submitted for EEEV RT-PCR were positive. Further history revealed that the affected emus were not in closely associated pens, the vitamin K treated emu died, and 7 of 12 emus had died within one week. The combination of clinical presentation, gross post mortem examination, and RT-PCR were consistent with a diagnosis of EEEV.

EEEV is one of the alphaviruses in the togavirus family. Wild birds are considered to be the reservoir host, and the disease is seen in areas with high mosquito populations. In 2003, there has been an increase in the number of horses diagnosed with EEEV infection at the AHL (see article this newsletter). Emus are particularly sensitive to EEEV and are presented because of acute mortality and terminal hemorrhagic diarrhea or emesis of bloodstained ingesta. **On post mortem examination of emus, lesions are viscerotropic rather than encephalitic as seen in other species**.

Eastern equine encephalitis cannot be treated, but some emus may respond to supportive therapy. Management is through control of the mosquito population, and it is possible to vaccinate using the commercial inactivated equine vaccine. **Vaccination of emus with the equine vaccine is considered off-label use and protection may not be adequate.**

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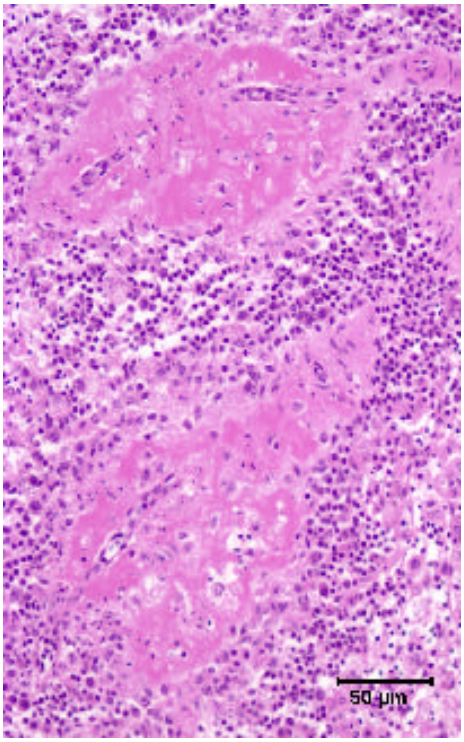


Figure 1. Marked fibrinoid necrosis of sheathed arterioles in the spleen of an emu with Eastern equine encephalitis.

Inclusion body hepatitis update

Davor Ojkic, Brian Binnington, Emily Martin *bbinning@lsd.uoguelph.ca*

Natural infection with most fowl adenoviruses (FAdV's) often causes no or only mild clinical signs. However, **inclusion body hepatitis (IBH) associated with FAdV infection affects 2-5 week-old broilers worldwide.** A variety of FAdV serotypes have been isolated from birds with IBH. It appears that the genotype, rather than the serotype, plays an important role in the pathogenicity of a particular FAdV strain.

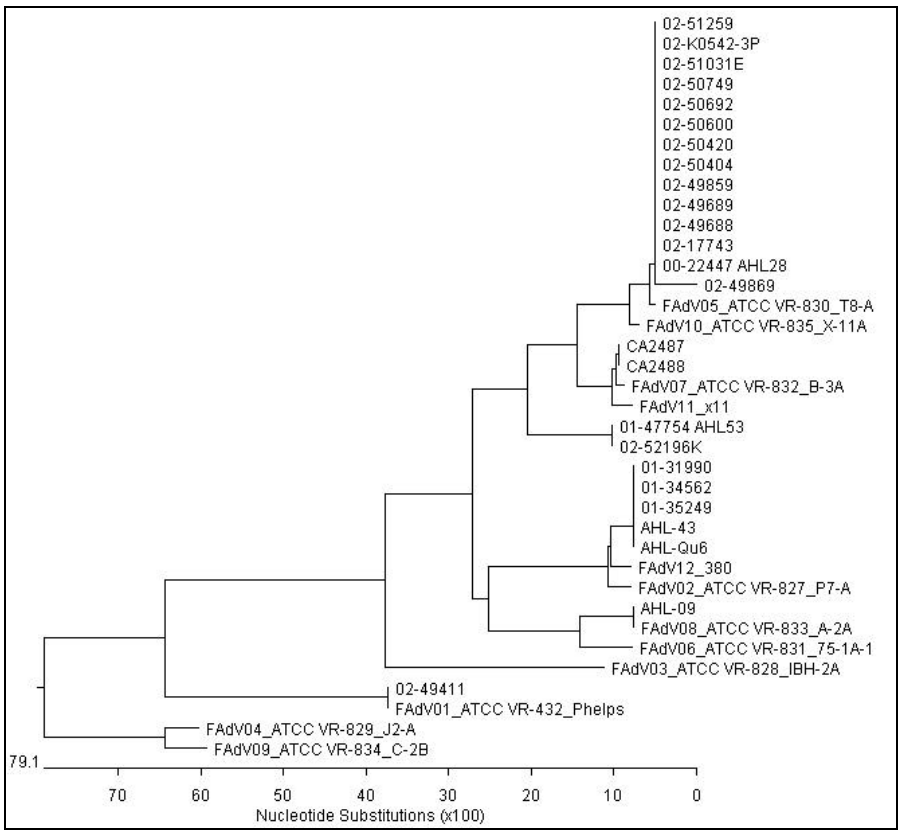
IBH is not exclusively limited to chickens; a turkey adenovirus has been implicated in hatchability problems in turkeys and was isolated from day-old turkeys with IBH. FAdV inclusions were found in chickens and quail with inclusion body ventriculitis in the USA. Gizzard erosions, pancreatitis and proventriculitis have been observed in chickens experimentally infected with FAdV's isolated from broilers with proventriculitis.

Hepatitis/hydropericardium syndrome (HHS) is an emerging disease described in Asia and South America and has been associated with FAdV-4. HHS affects broilers 3-5 weeks of age and can cause up to 75% mortality. Hepatic necrosis caused by FAdV-4 related viruses has also been described in psittacines and pigeons.

Diagnosis of FAdV infection is routinely done by a combination of histopathology, virus isolation and electron microscopy. Antibodies (Abs) to the group antigen can be detected by agar gel immunodiffusion, but it may be difficult to interpret the results since anti-FAdV Abs are frequently found in both sick and healthy chickens.

IBH has been occurring yearly in Ontario. An outbreak of IBH that occurred in November 2002 caused high mortality (up to 25%) in very young broilers (6-16 days). We introduced a new test for rapid genotyping of FAdV's, characterized viruses isolated from broilers during the outbreak, and compared them to prototype viruses and FAdV isolates associated with IBH outbreaks in previous years. The comparison was done by sequencing and phylogenetic analysis of the L1 loop of hexon protein amino acid (aa) sequences that gives an estimate of genetic relatedness between FAdV's. Hexon aa sequences from 12 field strains isolated during the 2002 outbreak were identical amongst themselves and 98.7% similar to FAdV-5. Only one previous field isolate (May 2000) had a hexon protein aa sequence identical to the year 2002 IBH viruses. Serological findings from affected broilers were also positive for FAdV-5 antibodies. Our ongoing work determined that other genotypes have been associated with IBH in previous years.

For more information about testing and IBH diagnostic approaches please contact Dr. Davor Ojkic at 519-824-4120, ext. 54524 or Dr. Brian Binnington at 519-824-4120, ext. 54550.



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Soike D, et al. Adenovirus infections in psittacines. Tierarztl Prax Ausg Klientiere Heimtiere. 1998;26:354-359.

Figure 1. Phylogenetic tree depicting the relationship between Ontario field FAdV's and selected reference viruses.

SWINE

Hind leg lameness and arthritis due to *Mycoplasma hyosynoviae* in grower pigs

Tony van Dreumel, Sameh Youssef, Pat McRaild, Beverly McEwen *tvandreu@lsd.uoguelph.ca*

A 2400-sow multi-site, all-in all-out by room system had an ongoing problem with acute onset lameness over a period of several months. **Affected pigs were approximately 2 months old when they developed acute non-weight bearing lameness of the hind legs.** Some pigs had swollen joints. Treatment rates of affected groups were 10-40% higher compared to regular treatments. Growth rate was also reduced.

Response to treatment with a combination of penicillin and Predef or Tylan and Predef was generally positive but variable. More recently, strategic treatment with lincomycin in feed resulted in a favorable response initially, but after the treatment was discontinued a high percentage of pigs became lame at a later date. Mortality was less than 1%.

Swabs and fresh synovia from stifle and hip joints, and formalin-fixed synovia of 2 affected pigs were submitted to the laboratory. Bacteriology yielded no growth, but culture of synovia on modified Friis medium yielded moderate numbers of colonies that fluoresced when stained with *M. hyosynoviae* FA conjugate (Fig. 1). Sections of joint capsules had subacute hyperplastic suppurative and lymphocytic synovitis.

Hind leg lameness and arthritis due to *M. hyosynoviae* has recently been reported in grower-finisher pigs in Denmark. Outbreaks of *M. hyosynoviae* arthritis tend to be sporadic (Table 1) but they may cause significant economic losses in large production units, as seen in this herd.

Routine bacteriology cultures will not grow mycoplasmas. Duplicate joint swabs should be submitted to the laboratory for routine bacteriology as well as mycoplasma cultures. Sections of joint capsules in formalin should also be included.

Reference

Nielsen EO, Nielsen NC, Friis NF. *Mycoplasma hyosynoviae* arthritis in grower-finisher pigs. J Vet Med A Physiol Pathol Clin Med 2001;48:475-486.

Table 1. *Mycoplasma hyosynoviae* isolates, cases and positive herds identified by AHL Mycoplasmology lab, 1998 – October 2003, less than half of the isolates were from joints

	1998	1999	2000	2001	2002	2003	Grand total
Number of isolates	12	10	4	11	13	5	55
Number of cases	7	6	4	4	7	3	31
Number of herds	7	6	4	4	6	2	29

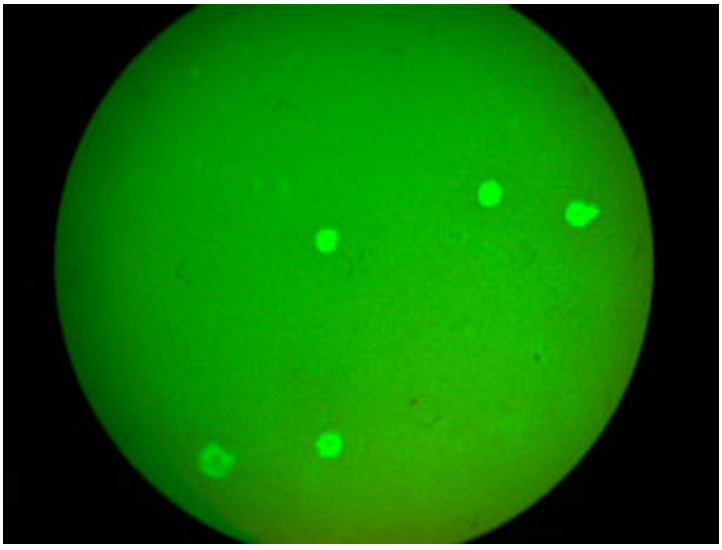

Fig. 1. Fluorescence of mycoplasma colonies on modified Friis medium stained with *M. hyosynoviae* FA conjugate.

Actinobacillus pleuropneumoniae and *Staphylococcus aureus* co-infection in 3 pigs with cellulitis

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Three 4-week-old pigs from a 2,500 head, nursery-to-finish barn were submitted to the AHL for diagnostic purposes. The sow herd was PRRSV- and *Mycoplasma hyopneumoniae*-free, as well as being serologically negative for *Actinobacillus pleuropneumoniae* serotypes 1 (9, 11), 5 (a, b) and 7 (4). These were the only pigs affected. Post-mortem examination revealed lesions confined to the head and neck in 2 of the pigs; lesions also extended onto the shoulder area in the third animal. There were multiple, randomly scattered, irregular areas of swelling, with ulceration, necrosis and/or epithelial hyperplasia over affected areas. Small proliferative and/or ulcerated areas were also present on the gums. Regional lymph nodes were enlarged, with pyogranulomatous pale yellow foci throughout. Diffuse edema accompanied the lesions seen in Figure 1.

A consistent microscopic feature was pyogranulomatous inflammation, both in the subcutaneous tissues as well as in affected lymph nodes. Club colonies containing gram-negative bacteria were identified within these granules. Electron microscopic examination of affected tissues failed to identify the presence of viral agents. Bacteriological examination identified the presence of large numbers of *A. pleuropneumoniae* along with small numbers of *Staphylococcus aureus.* An *A. pleuropneumoniae* isolate was forwarded to the Faculté de Médecine Vétérinaire, Service de Diagnostic, Université de Montréal, where the isolate was confirmed by PCR testing as *A. pleuropneumoniae* and serogrouped as serotype 12.

The skin supports the growth of commensal microorganisms, which have a protective role against pathogenic bacteria. Host immunity, microorganism adherence and virulence are intricately involved in cutaneous infection. *Staphylococcus aureus* is a well-known pathogen of the skin and is regularly seen in cellulitis, impetigo, folliculitis and furunculosis. Moreover, *S. aureus* produces toxins, which may trigger massive release of cytokines. *A. pleuropneumoniae* is considered to be a respiratory pathogen, although arthritis, endocarditis and abscesses may occur in individual animals. In primary outbreaks, abortions may occur, and middle ear infections have been diagnosed. **To our knowledge, skin co-infection of *A. pleuropneumoniae* and *S. aureus* have not been described and this is the first case of skin abscesses/pyogranulomas observed by AHL personnel involving these two microorganisms.** A toxin (ApxIVA) of *A. pleuropneumoniae* has been described as showing weak hemolytic activity and co-hemolytic synergy with the sphingomyelinase (beta-toxin) of *S. aureus.* Therefore, it is tempting to speculate some type of synergistic co-infection in these cases.

From January 1, 1997 to June 30, 2003, a total of 168 *A. pleuropneumoniae* isolates have been typed at AHL with 8 being non-typable, using antibodies against serotypes 1, 5 and 7 (Table 1).

Table 1. *A. pleuropneumoniae* serotypes isolated at the AHL

Serotype	No. of isolates
1	49
5	49
7	37
Poly-reacting strains*	20
12	1
Autoagglutinate	4
Non-typable	8

*strains that share more than 1 serotype (1,5; 1,5,7; etc.)

In specimens that have been submitted to the AHL, *A. pleuropneumoniae* serotypes 1, 5 and 7 predominate in Ontario. However, we are unaware of the presence and/or prevalence of other serotypes, which would be included in at least the 8 non-typable isolates.

In summary, the distribution of the lesions in all 3 pigs suggests that the initial entry point to the body was through the skin abrasions that occur during establishment of the hierarchical dominance in recently mixed animals. However, the source of *A. pleuropneumoniae* serotype 12 remains a mystery.

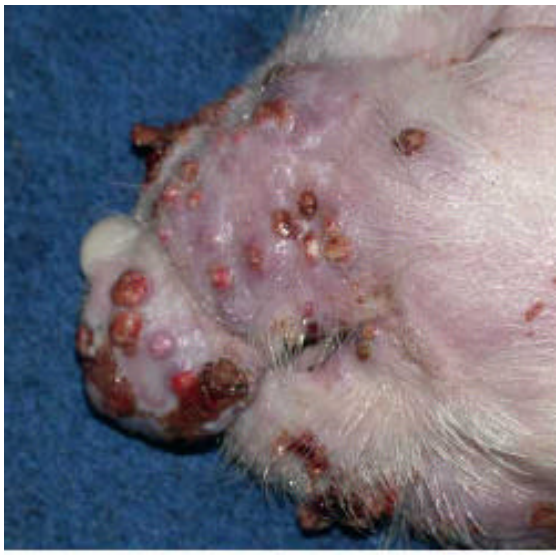

Figure 1. Pig snout with marked cellulitis and randomly scattered, irregular areas of swelling, with ulceration, necrosis and/or epithelial hyperplasia over affected areas.

HORSES

West Nile virus and EEE virus infection in horses in Ontario, 2003

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Viral neurological disease in Ontario horses was again an interesting clinical, diagnostic, and epidemiologic entity during the summer of 2003. Although total numbers of clinical cases remained small for a variety of purported reasons, including widespread vaccination programs and changes in weather patterns, Eastern equine encephalitis virus (EEEV) infection was more common than West Nile virus (WNV) infection.

From August to October, **3 cases of equine WNV infection were diagnosed at the Animal Health Laboratory;** these included 2 animals with positive WNV IgM ELISA (one of which is still alive) and one necropsy case in which WNV antigen was demonstrated by immunohistochemistry in foci of non-suppurative inflammation in brain. Of 8 additional necropsy cases from horses with neurological disease, none was positive for WNV using RT-PCR. Neither of the serologically positive horses had been vaccinated against WNV; the vaccination status of the animal submitted for necropsy is unknown. Five additional Ontario horses with confirmed WNV infection were diagnosed by positive IgM ELISA at other institutions during this same time period. Although there were numerous horses with positive WNV IgG ELISA, **the presence of anti-WNV IgG is indicative of previous exposure only and is not conclusive to establish recent infection**. Each of the WNV-infected horses originated from a different county, consisting of Peel, Halton, Niagara, Essex, Durham, Perth, Peterborough, and Stormont-Dundas-Glengarry counties. The total of 8 infected horses for the 2003 season is in sharp contrast to the prevalence of WNV infection in 2002, during which 107 positive equine cases were diagnosed in Ontario, including 70 at the AHL. Of the AHL cases, 22 were diagnosed at necropsy and 48 cases were diagnosed serologically.

> EEE virus made a dramatic reappearance in Ontario this summer

EEE virus made a dramatic reappearance in Ontario this summer. A similar increase in the incidence of EEEV was identified along the eastern seaboard of the United States and in some areas of the Midwest. **From late July to October 2003, EEE was diagnosed in 7 horses submitted for necropsy to the AHL.** These animals had positive RT-PCR results for EEEV nucleic acid in brain, in combination with histologic lesions of non-suppurative to mildly neutrophilic encephalitis. Two of these confirmed positive animals and 3 additional animals with probable EEEV infection also had high single-sample serum neutralization titers ($\geq$1:320) for anti-EEEV antibodies in tests conducted by CFIA. Of the 10 horses with confirmed and probable EEEV infection, 9 had not been vaccinated against EEEV; the vaccination status of one animal is unknown. The majority of these horses originated from eastern Ontario, with few animals from more western regions; counties of origin of positive and probable EEE cases include Lanark, Ottawa-Carlton, Leeds and Grenville, Stormont-Dundas-Glengarry, Simcoe, and Manitoulin. Prior to 2003, EEE was last diagnosed at the AHL in 1994.

Clinical signs reported in EEE virus-infected horses were very similar to those previously described in animals with WNV infection, and included sudden, rapid onset of pyrexia, ataxia, lethargy, depression, recumbency, seizures, and occasionally cranial nerve deficits. Affected horses ranged from 2.5 months to 10 years of age. The case fatality rate for EEEV-infected animals was high among Ontario horses, with death or euthanasia of all confirmed cases and one probable case (80%). Two of the horses with probable EEE survived, with complete recovery of neurologic function.

Diagnostic plan for horses with encephalitis

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Pathogens identified in Ontario that cause equine neurological disease include **rabies**, **West Nile virus** (WNV), **eastern equine encephalitis virus** (EEEV), **equine herpesvirus-1** (EHV-1), ***Sarcocystis neurona*** (equine protozoal myeloencephalitis, EPM), ***Halicephalobus (Micronema) deletrix***, and **bacteria**. In the past year, WNV has persisted in Ontario and EEEV has reappeared, affecting larger numbers of horses than in previous EEEV outbreaks.

To address identification of emerging equine pathogens in Ontario, the AHL has developed an immunoperoxidase test for WNV, PCR tests for both WNV and EEEV, and has implemented WNV serology within the past year. Through the use of these new diagnostic tests, the AHL was able to alert provincial and federal governments to new cases of EEE and WNV as they occurred this past summer.

(continued on page 47)

Diagnosis, horses, encephalitis - *from p 46*

Rule out rabies first, taking the usual precautions, and informing your CFIA District Office. If rabies has been ruled out or is unlikely, horses with acute encephalitis should be tested for WNV, EEEV and other pathogens.

For a live horse with neurological signs:

- WNV – single serum for IgM ELISA
- EEEV - acute & convalescent sera for virus neutralization (VN) *(CFIA will test unvaccinated horses only)*
- EHV-1 - acute & convalescent sera for VN
- EPM - serum and/or CSF for Western blot

Although too late to help the individual animal, an etiologic diagnosis will help horse owners by detecting new and emerging diseases in our equine population and will contribute to public health awareness when zoonotic pathogens are identified.

For a dead horse with neurological signs:
Remove and sagittally section entire brain; remove sections of thoracolumbar spinal cord.

- submit ½ brain to CFIA for **rabies**
- freeze 1 cm slice of each of rostral brain, medulla, and spinal cord for PCR (WNV, EEEV) and/or EHV-1 virus isolation, bacteriology
- fix remainder of brain and cord in formalin for histopathology, immunohistochemistry (IHC) for WNV, EEEV, EHV-1, EPM

OTHER SPECIES

Polyserositis and pyothorax in a group of mink associated with *Acinetobacter anitratus* infection

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Five dead mink with a 2-day history of a sudden increase in anorexia and mortalities were submitted from a mink farm for postmortem examination. On necropsy examination, the thoracic cavity of the mink was filled with dark-brown to red, serosanguineous fluid admixed with fibrinopurulent tags. The lungs were heavy with a resilient texture. A tentative diagnosis of bacterial pleuropneumonia was made. Mortalities stopped two days after a trimethoprim-sulfa antibiotic was added to the feed.

Large numbers of *Acinetobacter anitratus* were isolated in pure culture from pooled thoracic swabs and pooled lung samples. *Acinetobacter anitratus* was resistant to 50% of the 14 antimicrobials tested, but it was susceptible to trimethoprim-sulfa. On histologic examination, one or more of the following changes were present in all mink; severe fibrinosuppurative and hemorrhagic pleuropneumonia with large numbers of intralesional and intrahistiocytic bacterial rods, marked bacterial colonization of kidney and epicardium of the heart with mild neutrophilic infiltration, and severe necrofibrinous peritonitis. Serological testing (counterimmunoelectrophoresis) was negative for Aleutian disease virus, and no morbillivirus (distemper) was demonstrated on fluorescent antibody testing of lung.

***Acinetobacter* species are widespread environmental bacteria found in soil, water, sewage, food (including spoiled poultry meats), and on human skin as part of normal skin flora**. This microorganism is known for its ability to colonize various body sites, survive up to several months on most environmental and dry surface, and spread easily between patients. They are frequent causes of hospital-acquired infections, especially in immunocompromised individuals, and can result in serious infections due to their frequent resistance to multiple antimicrobials. They have been associated with a wide spectrum of human infections such as wounds, urinary and respiratory, bacteremia, and ventilator-associated pneumonia in intensive care unit patients. Hospital-acquired infections are frequently associated with direct contact with contaminated equipment; movement of this equipment or carriage on the hands of hospital workers are common causes of dissemination in hospital outbreaks. Aerosol spread has also been suggested in respiratory disease outbreaks. ***Acinetobacter* spp. infections have been reported in horses, dogs, cats and one source mentions bronchopneumonia in mink**.

A source of the *Acinetobacter* spp infection on this mink ranch was not determined. Contaminated feed ingredients or water are a possibility. The disease seemed to spread to mink in close proximity to affected mink. Direct contact with affected mink, contaminated equipment, cages or aerosol spread may have occurred. Although immunosuppressive agents such as Aleutian disease virus or distemper virus were not identified, stress-related factors as well as the presence of *P. aeruginosa* may have been important in this outbreak.

Reference

Bergogne-Berezin E, KJ Towener. *Acinetobacter* spp. as nosocomial pathogens: microbial, clinical and epidemiological features. Clin Microbiol Rev 1996;9:148-165.

COMPANION ANIMALS

Acute grape toxicosis in a dachshund

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A 1-year-old neutered male wire-haired dachshund developed acute onset of vomition, dehydration, paddling, head and body tremors after accidentally ingesting a small amount (estimated 1-2 clusters) of immature green Concorde grapes. The dog vomited a few of the grapes over a period of ~ 1 hr. He also became unaware of his surroundings, and failed to recognize and respond to the owner. He had a slight fever (38.5°C), increased pulse (160/min) and forced abdominal respirations when admitted. Hematology results indicated moderate dehydration, and serum biochemistry profile results were compatible with mild kidney and liver dysfunction.

Because the dog had vomited several times prior to admission, it was decided not to induce vomiting but to treat him with 150-200 ml of activated charcoal orally. He was given IV fluids 2x maintenance dose for the first 4 hr followed by 1.5x maintenance dose for the next 48 hrs. Urine output was measured over a 24-hr period to ensure fluid intake equaled output. He made a complete recovery.

There are only a few reports of grape/raisin toxicosis in dogs, and most of these have been recent. The toxic principle in the grapes, which causes mainly acute renal failure, has not been identified. All of the 10 cases reported by workers in Illinois had proximal renal tubule degeneration and/or necrosis of variable severity (1). Most dogs seem to like grapes and owners are often tempted to give them a few. **The purpose of this report is to alert veterinarians and owners to the fact that grapes/raisins are highly toxic to dogs, with potentially lethal consequences unless the animals are treated immediately.**

Recommended therapy includes induction of vomition, gastric lavage and administration of activated charcoal immediately after ingestion. This is followed by IV fluids for a minimum of 48 hrs or at least until hematological and serum profile values return to normal levels.

Reference

Morrow CK, Valli VE, Volmer PA. Renal pathology associated with grape or raisin ingestion in canines: 10 cases. Proc 46th Ann Mtg AAVLD. San Diego. 2003:128.

Show me some skin - obtaining useful information from skin biopsies

Zack, an 11-year-old, intact male mixed-breed dog, was presented with a 2-month history of alopecia, crusting, and pruritus involving the nose, ear margins, trunk, and footpads.

- 2 biopsies taken from the skin over the trunk demonstrated severe epidermal hyperplasia with infiltration of eosinophils around blood vessels in the superficial dermis.
- The histologic lesions were **most suggestive of ectoparasite infestation**, with sarcoptic mange as the primary differential diagnosis.
- The dog did not respond to therapy for ectoparasites or with antibiotics, and was re-examined 2 months later, with identification of similar lesions that had extended to involve axillae.

Turn to page 39 for the final diagnosis on Zack.

Are you on the lookout for FAD's?

This may seem to be an unusual question for a small animal practitioner, but it comes to mind because **the last 2 index cases of exotic Newcastle disease (END) in California (1998 and 2002/03) were first detected through companion animal clinic submissions.** These disease outbreaks started in 'game birds', and had devastating multi-million dollar effects on commercial poultry operations. Given the speed of international travel, the possibility of illegal importations, and possible close associations of game birds, psittacines, and commercial poultry, it behooves all veterinarians, including companion animal practitioners, to stay on the alert for foreign animal diseases.