UNIVERSITY of GUELPH LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

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TSE update - retooling to a rapid test

Davor Ojkic, Murray Hazlett

Big events are happening on the BSE/CWD/scrapie front, as the AHL, supported by the Ontario Ministry of Agriculture and Food, retools the TSE laboratory for the BioRad ELISA. This test should give more rapid turnaround than the traditional immunohistochemistry (IHC), which we have discontinued. We plan to have the new test in place and validated by September. This new test will be done by the immunology/serology workgroup under the direction of Dr. Davor Ojkic.

For cervid producers who need IHC for CWD herd certification programs, please send to the AHL as usual, we will block and prepare the first part of the testing, and forward it to the CFIA reference lab for the completion of the test. As the rules for acceptable tests change, it may be that the rapid test will become acceptable for certification purposes.

Welcome Dr. Slavic!

We are pleased to announce that **Dr. Đurđa Slavić** (pronounced Jurja Slavich) joined the AHL in August 2004, as our **veterinary bacteriologist**. Dr. Slavic earned her DVM degree in 1994 at the Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia. Upon graduation she spent two years in Croatia working in veterinary inspection.

In 1997 she enrolled in a Master's program in the Department of Pathobiology, OVC, investigating serological and biological properties of *Actinobacillus suis* lipopolysaccharides. Currently she is completing her Ph.D. thesis concerning the characterization of capsular polysaccharides of *A. suis* in the same department. She expects to defend her thesis this fall.

Quick notes:

- The **UofG telephone system** is currently being upgraded. Phones at 95 Stone Rd. (Toxicology) were switched to the new system in July, and phones in the main AHL lab within OVC are scheduled to be switched Sept. 29. All phone numbers will remain the same, and we expect minimal interruptions to service.
- **Please always record the age(s) of animals on submission forms**. This is particularly important in labs such as Bacteriology, where the setup for testing varies with the age of the animal.
- We will **email your reports** to you if you provide your email address - contact us at holiver@lsd.uoguelph.ca .
- You need not record the full address of a food animal owner on AHL submissions, BUT **we do require the postal code** to meet disease surveillance goals for OMAF.
- Our *Packing and shipping lab submissions* handout is available as a **color poster** for your shipping area, is on the Web at *http://www.uoguelph.ca/ahl/UsersGuide/UsersGuide.htm* , and is reprinted in the *AHL User's Guide and Fee Schedule*, pages 7 & 8. **Please submit fecal specimens in plastic screw-cap jars, NOT in rectal sleeves, Vacutainer tubes, or plastic bags** (all of these can leak and/or explode).

Methicillin-resistant *Staphylococcus aureus*: an emerging veterinary and zoonotic pathogen

J. Scott Weese *jsweese@ovc.uoguelph.ca*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen that is of tremendous concern in human medicine and is emerging as an important veterinary pathogen. **MRSA isolates are resistant to all beta-lactam antimicrobials (penicillins, cephalosporins) and usually are resistant to multiple other classes of antimicrobials, meaning infections may be very difficult to treat.** While originally regarded as strictly a nosocomial pathogen, MRSA infections are increasingly being reported in people with little or no contact with the healthcare system.

Recently, MRSA infection and colonization (carriage without clinical infection) have been identified in domestic animals, including horses, dogs, cats and rabbits. In a number of situations, people (owners, veterinary personnel) have been colonized with the same strain. Many small animal infections have been wound or post-operative infections, and some have been fatal. MRSA isolates obtained from these cases were consistent with the major human strains, supporting the **hypothesis that MRSA in small animals represents a 'spill-over' from the human population** and that MRSA originated from someone in the household or veterinary clinic.

The situation is different in horses, because **MRSA appears to be endemic in some sectors of the horse population.** One MRSA strain that likely originated in humans (but is uncommon) appears to be adapted for survival in horses and has accounted for almost all isolates obtained from horses and horse personnel. A recent study of horses on farms in Ontario and New York with a history of MRSA colonization identified MRSA in 4.7% of horses and 13% of equine personnel. MRSA can become endemic on farms and colonization rates can exceed 50%. Approximately 2.7% of horses admitted to the OVC Veterinary Teaching Hospital are colonized at the time of admission, and horses colonized with MRSA appear to have a much greater likelihood of developing clinical MRSA infection. Clinical MRSA infections have developed in a few healthy people working with colonized or infected horses.

Veterinarians should ensure that the laboratory they use tests for MRSA, that is, they check all *S. aureus* isolates for oxacillin resistance. All animals infected or colonized with MRSA should be considered a source of infection for humans and animals. Hospitalized animals should be isolated and handled with barrier contact precautions. Standard infection control practices such as hand hygiene should be emphasized. **Owners of MRSA-infected or colonized animals should be informed of the diagnosis and potential for zoonotic transmission.** Clusters of nosocomial infection or identification of ongoing cases should trigger an infection control investigation to determine the cause and appropriate intervention measures.

An active research program involving the University of Guelph, Mt. Sinai Hospital, and Vita-Tech Laboratories is underway to evaluate MRSA infection and colonization in animals throughout North America. *AHL*

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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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New real-time PCR tests available at the AHL

Hugh Cai, Patricia Bell-Rogers, Davor Ojkic *hcai@lsd.uoguelph.ca*

Beginning August 1, 2004, the AHL will add 3 new real-time PCR tests; ***Brachyspira hyodysenteriae* real-time PCR, *Brachyspira pilosicoli* real-time PCR** (both on fecal and intestinal samples), and **real-time PCR scrapie susceptibility genotyping** (prion gene codons 136, 154 and 171) on EDTA blood samples.

The AHL has been providing PCR test services since 1998. In the past 2 years, we have been developing and validating real-time PCR assays. Real-time PCR is a recently introduced technology that can detect PCR products during amplification (conventional PCR involves detecting PCR products after amplification using gel electrophoresis). In a real-time PCR instrument, PCR products are detected during each cycle of the PCR reaction by reading a fluorescent signal that has been incorporated into the product (Figure 1). **Compared to conventional PCR, real-time PCR is faster, offers more accurate quantitation and reduced risk of contamination because it is a closed-tube system**.

In conventional PCR, amplification of different concentrations of templates all reach log phase so that it is not possible to quantitate accurately the initial template by measuring the end PCR product. By monitoring the PCR reaction in real-time, the cycle number (threshold cycle) at which a template enters the log-linear phase can be determined. By comparing the threshold cycle of the target with that of standard controls, the initial template concentration can be determined. For example, in a field validation project (supported in part by Ontario Pork), we found that some healthy carrier pigs shed 1,000 *Brachyspira hyodysenteriae* cells per 0.2 g of feces, while suspected swine dysentery pigs shed 100-10,000 times more. One can foresee that the ability to quantitate the pathogen load will help to differentiate infection from disease.

After the real-time PCR is completed, the melting temperature (Tm) of the double-stranded PCR products can be determined by measuring the fluorescence intensity under different temperatures. Since the Tm of a DNA duplex is determined by its nucleotide composition, Tm profiles can be used for bacterial strain typing or genotyping. The real-time PrP genotyping for scrapie susceptibility is based on this principle. For this assay, we will use a commercial kit (TIB Molbiol, Adelphia, NJ) that has been validated by testing DNA of different genotypes, including AARRRR, AARRQH, AARRQQ, AARHRQ, AARRRQ, AVRRQQ, AVRRQH, AARRRH, and AARHQQ. At the AHL, the kit was tested 4 times against genotypes AARHQQ, AARRQR, AVRRQQ, AARRRR, AVRRQR, AARRQQ, which were kindly provided by CFIA, Ottawa. In addition, the kit was used to test 6 field samples in parallel with sequencing methods. No non-specific reactions were found.

The fee for *Brachyspira hyodysenteriae* or *Brachyspira pilosicoli* real-time PCR is $24 for each sample, $36 for both tests for each sample. The fee for real-time PCR scrapie susceptibility genotyping is $36 for three codons (codons 136, 154 and 171). For further information, please contact Dr. Hugh Cai (ext. 54316) or Patricia Bell-Rogers (ext. 54086).

Real-time RT-PCR tests for **West Nile virus**, **avian influenza virus** and **Eastern equine encephalitis virus** are also available at the AHL. For more information, contact Dr. Davor Ojkic (ext. 54524). *AHL*

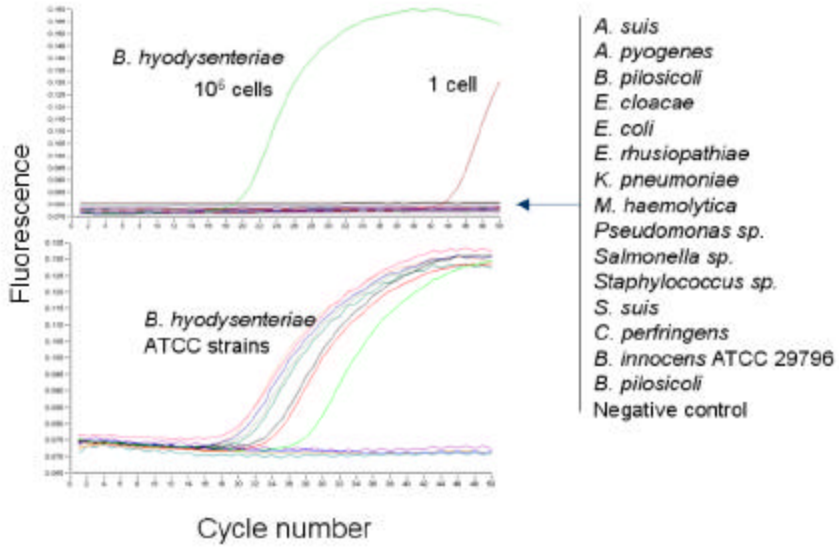

Figure 1. Examples of two real-time PCR runs, demonstrating the specificity of *B. hyodysenteriae* real-time PCR. In **run 1** (above), none of the organisms listed on the right generated a positive PCR signal i.e., the signal is specific to *B. hyodysenteriae*. In **run 2** (below), all of the *B. hyodysenteriae* strains tested amplified.

AHL Lab Reports

CATTLE

Bovine abortion update, 1998-2004

Beverly McEwen, Susy Carman *bmcewen@lsd.uoguelph.ca*

***Neospora* spp. continues to be the single pathogen most frequently identified in bovine fetuses submitted to the AHL for gross and/or histological examination** (Table 1). The frequency of abortions due to BVDV, *A. pyogenes,* and fungi have increased since 2000/2001. The reason for the increase in abortions due to *A. pyogenes* is not known; all fetuses were submitted from different herds.

The number of bovine abortions submitted for examination has decreased since 1998/1999 and currently represents 4.2% (n = 127) of identified herd level submissions to the AHL. The diagnostic rate was 100% for herds submitting 3 or more abortions, 68% for herds submitting 2 abortions and 37% for herds submitting only one abortion. **The ability to determine an etiologic diagnosis depends upon the number of submissions from an affected herd and the quality and type of specimens submitted. Submission of an entire fetus and placenta increases the diagnostic rate.**

Detailed information on appropriate sample submission is available in the 2004 AHL User's Guide & Fee Schedule and AHL Newsletter "Bovine abortion diagnostics", March 2002. *AHL*

Reference

DeLay, J. Bovine abortion diagnostics: Maximum benefit for your client's dollar. AHL Newsletter, 2002;6(1):4.

Table 1. Bovine abortion cases submitted to the Animal Health Laboratory, 1998 - 2004, fiscal years

Selected etiologic diagnoses # cases /(%)[1]	98/99	99/00	00/01	01/02	02/03	03/04
Neospora spp.	53 (14%)	58 (15%)	47 (19%)	36 (12.5%)	17 (8.3%)	21 (10.9%)
Placentitis, etiology not identified[2]	65 (17%)	58 (15.4%)	63 (25.4%)	43 (15%)	31 (15.2%)	21 (10.9%)
Arcanobacterium pyogenes	11 (2.9%)	14 (3.7%)	9 (3.6%)	8 (2.1%)	4 (2.0%)	13 (6.7%)
Bacterial abortion - other[3]	31 (8.1%)	29 (7.7%)	44 (13.4%)	24 (8.2%)	28 (14.0%)	12 (6.1%)
Mycotic abortion	14 (4%)	4 (1%)	4 (1.6%)	7 (2.4%)	7 (3.4%)	9 (4.7%)
Bovine viral diarrhea virus (BVDV)	8 (2.1%)	20 (5.3%)	8 (3.2%)	5 (1.7%)	6 (2.9%)	8 (4.1%)
Bovine herpesvirus type 1 (IBRV)	1 (0.3%)	28 (1%)	9 (3.6%)	7 (2.4%)	9 (4.4%)	6 (3.1%)
Bacillus licheniformis	5 (1.3%)	4 (1.1%)	5 (2%)	4 (1.4%)	4 (2.0%)	6 (3.1%)
Listeria monocytogenes	4 (10%)	5 (0.3%)	2 (0.8%)	6 (2.1%)	1 (0.5%)	3 (1.6%)
Ureaplasma spp.	9 (2%)	8 (2%)	12 (4.8%)	10 (3.5%)	14 (6.9%)	2 (1.0%)
Leptospira sp.	5 (1.3%)	1 (0.3%)	1 (0.4%)	1 (0.3%)	1 (0.5%)	2 (1.0%)
Coxiella burnetii	0	0	0	0	2 (1.0%)	0
Mycotic - *Candida* sp./yeast[4]	0	5 (1%)	5 (2.0%)	0	0	0
No significant lesions, etiology not identified	157 (41%)	151 (40%)	104 (41.9%)	131 (45.6%)	70 (34.3%)	74 (38.3%)
Total abortions submitted	**383**	**376**	**248**	**292**	**203**	**196**

[1] Pathology cases. Numbers represent diagnoses; more than one diagnosis may be present for a single abortion.
[2] Previously included with idiopathic abortions. Etiology possibly not identified as not all submissions requested bacterial, *Mycoplasma spp.* or virus culture.
[3] Includes *Escherichia coli, Salmonella spp., Staphylococcus spp., Haemophilus somnus, Streptococcus spp., Actinobacillus spp.,* and those with lesions compatible with bacterial abortion .
[4] Previously included with mycotic abortion.

POULTRY

Chronic osteoarthritis with amyloid deposition in layer chickens

Brian Binnington, Elizabeth Black *bbinning@lsd.uoguelph.ca*

A flock of leghorn chickens had experienced ongoing culling for lameness since placement. At 36 weeks of age, birds were submitted to a veterinary clinic for examination. The leg joints of these birds were swollen, with fibrosis around the joints. There was yellow/orange discoloration of tendon sheaths and periarticular tissues, pitting of articular cartilage with cavitations into the underlying bone, and serous to creamy exudates in some joints. Based on the gross necropsy appearance of the joint lesions in these birds, **amyloid arthropathy was one of the differentials** considered by Dr. Black. Bacterial cultures at the clinic did not yield significant bacteria, and samples were submitted to the AHL for serology and histopathology. Reovirus ELISA titers on 10 sera were low (G mean 1079) and *Mycoplasma synoviae* (MS) serum plate agglutination (SPA) tests were negative.

At 38.5 weeks of age, 4 live birds were submitted to the AHL for necropsy. Gross lesions were present in one or more of the leg joints that were similar to those seen in the birds at 36 weeks. Some of the joints also had a thickened joint capsule with villous proliferation (Fig. 1A.). Small numbers of *Enterococcus faecalis* were isolated from a hock joint of one bird. Four sera had reovirus ELISA titers (G mean 3765) and negative SPA and hemagglutination inhibition tests for MS and *Mycoplasma gallisepticum* (MG). No viruses were isolated from tendons utilizing SPF embryonated chicken eggs and cell cultures.

Microscopic lesions were similar in both submissions. Fibrosis of periarticular tissues and chronic active synovitis with accumulations of heterophils, lymphocytes and plasma cells were present in the tendon sheaths and joint capsule. Perivascular lymphocyte and plasma cell accumulations were present in the peritendinous connective tissues. Villous proliferation of the synovial membrane of the joint capsule was evident in some joints. Accumulation of fine fibrillar eosinophilic material was present in the joint capsule and periarticular fibrous tissues. This material was considered to be amyloid because it stained red-brown with Congo red and appeared apple green when viewed with polarized light. No amyloid deposits could be identified in visceral organs. Articular cartilage degeneration (fibrillation), erosion and ulceration with complete perforation of the articular cartilage were present. Areas of necrosis with fibrin, heterophils and macrophages were present in the subchondral (epiphyseal) bone. Some of the inflammatory foci in the epiphysis were cystic with a surrounding wall of fibrous tissue (Fig. 1B).

Amyloid arthropathy has been described most frequently in brown-egg laying birds in association with *Enterococcus faecalis* and less frequently *Mycoplasma synoviae* infections. It has been reported in broiler breeders, most frequently associated with *Staphylococcus aureus,* and rarely in white leghorn-type chickens in experimental infections with *Enterococcus faecalis* and in a field case of *Mycoplasma synoviae*. The arthritic lesions are more severe in the brown-type birds. Experimentally, inoculation of young chicks with arthritogenic strains of *Enterococcus faecalis* can lead to arthritis with amyloid deposition, however, it would appear that vertical, transovarian or transoviductal infections are not significant methods of chick infection. Experimental infection of brown layer chickens with arthropathic and amyloidogenic *Enterococcus faecalis* strains can consistently result in amyloid arthropathy. Cases of amyloid arthropathy do occur in hens during production, which suggests the existence of other routes of infection and/or predisposing factors. The natural routes of infection are not found in most field cases of amyloid arthropathy.

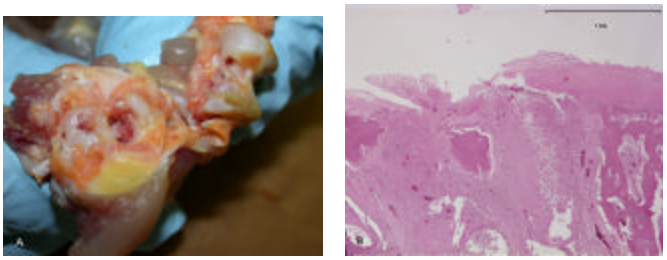

Figure 1A. Periarticular fibrosis and joint capsule thickening associated the yellow/orange deposits of amyloid. Severe cartilage ulceration and perforation. 1B. Degeneration and ulceration of articular cartilage. Chronic inflammation, fibrosis and cyst formation in the epiphysis .

The birds in this affected flock experienced a 9% mortality/culling loss during the production cycle. Although these birds are phenotypically white layers that lay white eggs, they do have brown layer bird genetics in their lineage. As chicks, the birds in this flock experienced increased culling due to foot and leg trauma. **It is likely that *Enterococcus faecalis* was a significant cause of the chronic infections in these birds.** There was no serological evidence of prior *Mycoplasma synoviae* infection. ELISA antibody titers to reovirus were present but no virus could be isolated. Experimental reovirus infections have not resulted in amyloid deposition. The role of reoviruses in this joint disease is undetermined. *AHL*

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Crespo R, Shivaprasad HL. Amyloidosis. *In:* Diseases of Poultry, 11th ed., Saif YM, et al., eds., Iowa State University Press, Ames Iowa. 2003:1058-1060.

Landman WJM. Amyloid arthropathy in chickens. Vet Quart 1999;21:78-82.

SWINE

Summary of PRRS virus strains in Ontario

Gaylan Josephson, Susy Carman, Hugh Cai *gjosephs@lsd.uoguelph.ca*

PRRS has had a significant economic impact on the swine industry, not only in Ontario but also worldwide. It is important that an accurate diagnosis be made prior to instituting control/preventive measures.

Several diagnostic testing procedures have been added to our arsenal during the past few years, but much has yet to be learned about the virus. Serology (PRRSV IDEXX ELISA) has been used extensively, but there are inherent difficulties in differentiating field vs. vaccine virus strain titers. The use of molecular techniques has improved our diagnostic capabilities.

From Jan. 1, 1998 to June 30, 2004, PRRS virus has been identified by polymerase chain reaction (PCR) testing in samples from 703 cases originating from herds in Ontario (Table 1). As expected, **PRRSV was identified most frequently during the winter months**. This is in agreement with statements made by practitioners about the incidence of PRRS. The apparent increase in PRRSV identifications that began in September 2003 was due to increased requests by practitioners for PCR and RFLP testing on serum samples submitted as part of routine PRRSV monitoring programs.

Of the 575 of 703 submissions in which a history was included, **the most frequent reason for submission was respiratory problems (51.3%);** pneumonia and diarrhea were mentioned in 9.1%, and pneumonia and arthritis in 3.5% of submissions. Other histories included: general unthriftiness with increased mortality (14.3%), reproductive problems (13.6%), diarrhea (5.6%), and arthritis (2.3%).

Using restriction fragment length polymorphism (RFLP) techniques, 39 different RFLP cut patterns were identified. In addition, 8 strains could not be classified using primers designed to evaluate open reading frame 5 (ORF5), with a capability of producing either a 716 bp or 933 bp product. Using restriction enzymes *Mlu*I, *Hinc*II, and *Sac*II, a definitive cut pattern could not be identified on 4 occasions with the *Hinc*II enzyme, and 4 with the *Sac*II enzyme. However, RFLP cut patterns were identified from these premises on subsequent submissions.

Of the 39 different RFLP cut patterns, the pattern 2-5-2 (found in ResPRRS) was identified 155 times (27.9% of all PRRSV strains whose cut pattern was identified). Intermediate strains (those differing in only 1 cut from the vaccine strain, e.g., 2-6-2 or 1-5-2) were identified on 42 occasions (7.6%). The other main cut patterns obtained were 1-7-2 (8.3%), 1-3-2 (6.8%), 1-3-4 (6.7%), 1-2-4 (5.8%), 1-4-2 (3.6%), 1-6-2 (3.4%), 1-2-2 (3.1%), and 1-1-4 (3.1%). Occasionally, some strains cannot be typed using either the 716 or 933 bp primer sets. In these cases, the nucleic acid that is obtained can be sequenced and this sequence utilized to determine RFLP patterns.

Gene sequencing along with gene sequence analysis (which includes a sequence distance report and a phylogenetic tree and a computer-generated predicted RFLP pattern) is available at the AHL, and is being used with increasing frequency. At present, we have sequenced approximately 145 strains. When compared to another strain, ≥98% homology suggests that the 2 strains are closely related. Thus, gene sequencing can be used to compare 2 or more strains, and is of particular value in epidemiological studies. Results from these comparisons provide valuable information to the veterinarian about the source of the virus, and may suggest that biosecurity protocols need to be strengthened. It is hoped that, with increased use of this technique, important information concerning the source of an outbreak can be determined, e.g., did the virus enter the herd via infected animal introduction, or was it due to area spread? *AHL*

Table 1. Number of PRRSV strains identified at the AHL by PCR, by month and year

	1998	1999	2000	2001	2002	2003	Jan-June 30, 2004	TOTAL (average)
January	3	6	7	8	16	11	28	79 (11.3)
February	8	10	15	6	9	11	20	79 (11.3)
March	10	20	11	8	9	14	22	94 (13.4)
April	9	12	17	3	9	12	15	77 (11.0)
May	5	7	11	6	7	10	12	58 (8.3)
June	6	3	5	6	7	9	17	53 (7.6)
July	6	2	4	9	2	5	-	28 (4.7)
August	3	6	3	11	3	2	-	28 (4.7)
September	5	14	4	2	8	10	-	43 (7.2)
October	9	6	3	7	9	12	-	46 (7.7)
November	9	6	11	7	10	16	-	59 (9.8)
December	10	5	4	12	7	21	-	59 (9.8)
Total	**83**	**97**	**95**	**85**	**96**	**133**	**114**	**703**

HORSES

Hemopericardium and suspected hypothyroidism in two stillborn Standardbred foals

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Two stillborn male Standardbred foals (different sires, dams, and farms of origin) had severe hemopericardium resulting from ~1 cm transverse tears of the pulmonary artery above the heart base. Histopathology revealed separation of tunica media fibers with faintly basophilic substance in the pulmonary arteries of both foals.

Thyroid glands of both foals appeared normal grossly, but histopathology was consistent with hypothyroidism, with most follicles containing proliferating epithelial cells and no colloid.

There are several references on dissecting aortic aneurysms in hypothyroid human adults, but none in prenatal or neonatal humans or in animals. In one study, 22% of 101 patients with aortic dissecting aneurysms were hypothyroid - the authors suggest that hypothyroidism may interfere with glycosaminoglycan metabolism, thereby causing a weakening of arterial walls.

The thyroids of both foals appeared normal grossly - it is important to examine thyroids histologically in all submissions from perinatal deaths. This case also suggests a relationship between prenatal hypothyroidism and pulmonary arterial ruptures in these foals. *AHL*

Reference

Rosenmann E, Yarom R. Dissecting aneurysm of the aorta and hypothyroidism. Isr J Med Sci 1994;30:510-513.

Mycobacterium paratuberculosis enteritis and hepatitis in a miniature donkey

Murray Hazlett, Robert Henderson, Claude Turcotte *mhazlett@lsd.uoguelph.ca*

An 18-month-old miniature donkey developed intermittent inappetance with bouts of unformed feces and abdominal discomfort over a 4-week period. Rectal prolapse occurred periodically during times when stool was soft. Upon death, it was submitted to the AHL. Necropsy revealed a 180° rotation of the stomach with bloat. Liver had an accentuated lobular pattern, and both small and large intestinal content was watery and green. The distal 10 cm of rectum was severely congested. Microscopically, liver had severe periportal neutrophilic and histiocytic inflammation, with fine basophilic stippling seen in many histiocytes. Similar inflammation was seen in lamina propria and submucosa of colon. Acid-fast stains of both organs revealed large numbers of intracytoplasmic acid-fast bacilli in histiocytes in both liver (Fig. 1) and intestinal sections.

Samples were submitted to the Mycobacterial Diseases Centre of Expertise, Canadian Food Inspection Agency laboratory in Ottawa, Ontario, and identified as *Mycobacterium paratuberculosis (Mycobacterium avium* subsp. *paratuberculosis)* .

A source for the organism was not determined. The donkey was housed with another miniature donkey, as well as a llama and alpaca in a paddock. The llama and the second donkey also developed intermittent inappetance and unformed feces, and occasional acid-fast bacilli were seen in feces of the llama, however both of these animals recovered spontaneously. The alpaca died of unrelated causes. Other animals that had been on the farm were clinically unaffected (two horses and two miniature goats).

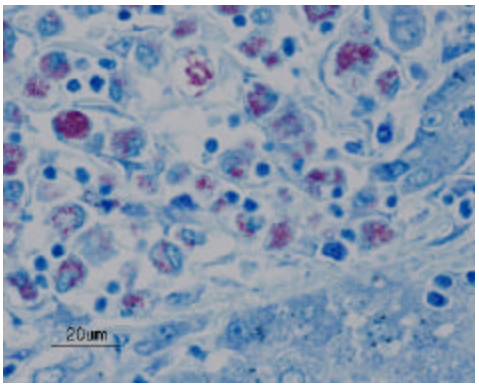

Figure 1. Acid-fast stain of granulomatous inflammation in hepatic portal regions with *Mycobacteria paratuberculosis* in macrophages.

***M. paratuberculosis* is an uncommon finding in horses, however disease has been produced experimentally**. The bacterium has been shown to be capable of replicating and being transmitted to other horses. A presumptive case of paratuberculosis has been reported in a Sicilian ass, however most of the equine mycobacteria reports in the literature are of *Mycobacterium avium* complex. *AHL*

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Larsen MS, Moon HW, Merkal RS. Susceptibility of horses to *Mycobacterium paratuberculosis*. Am J Vet Res 1972;33:2185-2189.

COMPANION ANIMALS

Five-year retrospective necropsy survey of tumors in dogs

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Neoplasia is a common cause of death in dogs, and breed predisposition for the development of neoplasia is well established. From 1998-2003, neoplasia was confirmed in 492/2758 (17.8%) of dogs necropsied at the AHL. When age was given, dogs with tumors were significantly older (mean age 8.7 yrs; median age 9 yrs) than dogs without tumors (mean age 5.4 yrs; median age 5 yrs). There were 132 breeds affected, as well as mixed-breed dogs.

Tumors were most frequently identified in Golden and Labrador retrievers, German shepherds, Rottweilers and Cocker spaniels (Table 1). Golden retrievers, German shepherds, Cocker spaniels and Standard Poodles with tumors at necropsy were over-represented compared to the overall submission rate of specimens from these breeds to the AHL.

The proportional mortality rate (PMR) indicates the cause of death due to tumor vs. no tumor in individual breeds. **As expected, the PMR was greatest in Boxers, followed by Golden Retrievers, Labrador Retrievers, Standard poodles, Rottweilers and Siberian huskies**. There was considerable breed variation regarding the diversity of tumors occurring in each breed, especially in the retrievers (Table 1).

For all breeds, including mixed-breed dogs, the most common malignant tumors identified were: hemangiosarcoma (19%), lymphoma (14%), osteosarcoma (5%), malignant histiocytosis (4%), unidentified sarcomas (3%), transitional cell carcinoma (2%), pheochromocytoma (2%), unidentified carcinoma (2%), pulmonary carcinoma (2%), unidentified round cell tumor (2%), mammary carcinoma (2%), and intestinal carcinoma (2%). *AHL*

Table 1. Frequency of tumors, proportional mortality, number of tumors and most common tumors identified in dogs necropsied at the AHL, 1998-2003

Breed (n = total number dogs with tumors/ total necropsied)	% of all breeds with tumors	Proportional mortality due to tumors (%)	# of tumor types identified in breed	Most common tumors (type/% of all tumors in breed)[2]
Golden retriever (n = 68/228)	14.0[1]	29.8	26	Hemangiosarcoma (35%), lymphoma (13%), unidentified sarcomas (6%), osteosarcoma (6%) malignant histiocytosis (6%), thyroid carcinoma (3%), unidentified carcinoma (3%)
Labrador retriever (n = 44/168)	9.0	26.2	23	Hemangiosarcoma (14%), osteosarcoma (9%), malignant histiocytosis (9%), lymphoma (9%), mast cell tumor (7%), thymoma (5%), pulmonary carcinoma (5%) meningioma (5%) lymphoid leukemia (5%), unidentified carcinoma (5%)
German shepherd (n = 25/147)	5.0[1]	17.0	16	Hemangiosarcoma (28%), osteosarcoma (12%), lymphoma (8%)
Rottweiler (n = 23/96)	5.0	24.0	12	Osteosarcoma (26%), unidentified sarcomas (13%), lymphoma (13%), round cell tumor (9%) malignant histiocytosis (9%)
Cocker spaniel (n = 13/73)	4.8[1]	17.8	11	Lymphoma (23%)
Boxer (n = 12/39)	2.5	30.8	10	Lymphoma (17%), meningioma (17%)
Doberman pinscher (n = 12/109)	2.3	11.0	7	Hemangiosarcoma (17%), lymphoma (17%), osteosarcoma (17%), pulmonary carcinoma (17%)
Standard poodle (n = 10/40)	1.8[1]	25.0	9	All tumors were represented only once
Siberian husky (n = 9/39)	1.4	23.1	6	Hemangiosarcoma (44%)
Collie (n = 7/46)	0.8	15.2	6	Thyroid adenoma (28%)

[1] over-represented compared to overall submission rate to the AHL
[2] number of tumors >1