UNIVERSITY of GUELPH
LABORATORY SERVICES

Animal Health Laboratory

AHL Newsletter

Canada Post Publications number - 40064673

Volume 14, Number 1, page 1 **March, 2010** ISSN 1481-7179

In this issue:

AHL introduces new submission forms!

Jim Fairles, Amanda Budd

- We are now providing new NCR (no carbon required) submission forms, **please replace any old AHL forms that may still be in your clinic**.
- You will now be able to keep an NCR duplicate of the submission form at your clinic. If you do not need or wish NCR forms, these are also available as single page forms.
- Because the forms are NCR, we will be using a **clinic sticker system** in place of custom forms. For those who use customized forms, you will receive sheets of stickers with your forms, with the clinic #, address, phone, fax number included (and veterinarian if desired). We ask that you fill in the veterinarian's name, and any additional information required below the sticker, directly on the form.
- All test requests are now on only one-side. Details of common tests are available on the back of each form. If a test is not present on the form, please refer to our User's Guide and Fee Schedule. The Fee Schedule (password protected under "Client login"– call or email for details) and new forms are currently on our website at: http://www.ahl.uoguelph.ca under "Where and how to submit specimens".
- Please feel free to give us your input on the new submission forms as we are trying to make them as user friendly as possible. **Please ask us about customized forms, on-line access to results, and on-line submissions as well.**
- For our large animal practitioners, a few changes will be apparent on the forms. **Demographic information is becoming increasingly important in the surveillance of animal disease**. We are asking practitioners to fill out this critical information, including farm municipality, CCS #, and postal code (explained on the back of the forms).

Lab profile - AHL Bacteriology

- Full service, proficiency-tested, accredited lab (AAVLD, ISO 9001-2008)
- In-house expertise - **Durda Slavic**, DVM, PhD, Veterinary Bacteriologist
  Hugh Cai, DVM, MSc, DVSc, Veterinary Microbiologist
- 17 full-time experienced technical staff in Bacteriology/Mycoplasmology/Molecular Biology

* Routine aerobic and anaerobic cultures for isolation and identification of bacterial and fungal pathogens, including *Mycoplasma* and *Ureaplasma*; individual cow and bulk tank milk culture.
* Antimicrobial susceptibility testing (MIC and disk diffusion).
* Radiometric culture-based detection of *Mycobacterium paratuberculosis* (agent of Johne's disease).
* PCR (polymerase chain reaction) -based tests for identification of slow growing, and hard-to-culture bacteria, e.g., *Mycobacterium paratuberculosis*, *Brachyspira* spp., *Lawsonia intracellularis, Coxiella burnetii.*
* 16S rRNA sequencing for bacterial speciation and PCR grouping of bacterial pathogens, e.g., enterotoxigenic *E. coli.*
* Mouse inoculation test for botulinum toxin detection.
* Routine screening of environmental and poultry samples for *Salmonella* spp. according to the Ontario Hatchery and Supply Flock Policy and European Union requirements.
* ELISA-based detection of *Clostridium perfringens* and *C. difficile* toxins.
* FA test and ELISA for *M. hyopneumoniae* antigen or antibody; FA test for *Mycoplasma* speciation.

For full list of tests, please see our Fee Schedule, or email Dr. Slavic dslavic@uoguelph.ca or Dr. Cai hcai@uoguelph.ca

Third call for proposals for Animal Health Strategic Investment – 9 new projects approved! *Jane Gaviller-Fortune*

A third call for proposals went out for Animal Health Strategic Investment (AHSI) projects in October 2009 and between November and January, nine new projects were approved for funding. This brings the total number of AHSI projects to 31!

The AHSI project was developed to support advances in 3 areas: 1) developing tests for emerging pathogens, 2) enhancing animal health surveillance, and 3) improving emergency and business-continuity planning. The following outlines the new research projects:

Proj. ID	Project lead Project team members	Project title	Project term
09-23	Bob Friendship, OVC George Charbonneau, Janet MacInnes, Jeonghwa Park, Zvonimir Poljak, Durda Slavic	Ear tip necrosis in swine	1.5 yr
09-24	Michele Guerin, OVC Agnes Agunos, David Léger, Scott McEwen, Rachel Ouckama, Cynthia Philippe, Richard Reid-Smith, Babak Sanei, Durda Slavic	Surveillance of *Salmonella* and antimicrobial resistant enteric bacteria in broiler breeder hatching eggs in Ontario - a pilot study	2.5 yr
09-25	Brent Hoff, AHL Luis G. Arroyo, Dan Kenney, Helen Kocmarek, Beverly McEwen, Kristiina Ruotsalo	Validation of serum amyloid A (SAA) an acute phase protein as marker of inflammation in horses	1 yr
09-26	Paula Menzies, OVC Jocelyn Jansen, Andria Jones	Prevalence of *Coxiella burnetii* infection in sheep flocks and goat herds in Ontario and their farm workers	2.5 yr
09-27	Éva Nagy, OVC Marina Brash, Laszlo Zsák	Surveillance for enteric parvoviruses in poultry – a pilot study	1 yr
09-28	Zvonimir Poljak, OVC Susy Carman, Enrique Castro, George Charbonneau, Robert Friendship, Durda Slavic	Risk-based surveillance of respiratory infections in growing pigs	1 yr
09-29	John Prescott, OVC Vicki Nowell, Valeria Parreira, Durda Slavic	Identification of novel toxin gene(s) associated with type A *Clostridium perfringens*-associated hemorrhagic abomasitis of calves in Ontario	1.5 yr
09-30	Karen Shearer, Fleming College Doug Campbell, Josepha DeLay, Davor Ojkic	Genotyping and detection of *Leptospira* spp. in wildlife reservoir hosts in Ontario through comparison of immunohistochemical and polymerase chain reaction genotyping methods	3 yr
09-31	Michele Guerin, OVC and Zvonimir Poljak, OVC Rob Deardon, Davor Ojkic	Case series of pandemic H1N1 in Ontario turkey breeder flocks	2.5 yr

AHL Newsletter
March, 2010 - Volume 14, Number 1
Editor: **Grant Maxie,** DVM, PhD, Diplomate ACVP
Editorial Assistant: **Ms. Helen Oliver**

The *AHL Newsletter* is published quarterly (March, June, September, December) by the Animal Health Laboratory, Laboratory Services Division, University of Guelph.

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

Mailing address & contact information:
(**please return all undeliverable Canadian addresses to**:)
Animal Health Laboratory
Laboratory Services Division, University of Guelph
Box 3612, Guelph, Ontario, Canada N1H 6R8
Phone: (519) 824-4120 ext. 54538; fax: (519) 821-8072
Email: holiver@uoguelph.ca

ISSN 1481-7179

Canada Post Publications number - 40064673

Contributors to this issue:

From the Animal Health Laboratory:

Marina Brash, DVM, DVSc, Diplomate ACVP
Amanda Budd, BSc
Hugh Cai, DVM, MSc, DVSc
Susy Carman, DVM, PhD
Josepha DeLay, DVM, DVSc, Diplomate ACVP
Jim Fairles, DVM, MBA
Jane Gaviller-Fortune, RQAP-GCP
Li Ge, MSc
Murray Hazlett, DVM, DVSc, Diplomate ACVP
Beverly McEwen, DVM, MSc, PhD, Diplomate ACVP
Davor Ojkic, DVM, PhD
Kristiina Ruotsalo, DVM DVSc, Diplomate ACVP
Durda Slavic, DVM, PhD
Maria Spinato, DVM, DVSc, Diplomate ACVP, MBA
Margaret Stalker, DVM, PhD, Diplomate ACVP

Other contributors:

John Barta, PhD; **Maged Gomaa**, BVSc, MVSc PhD; **Michael Taylor**, DVM; Ontario Veterinary College
Bryan Hicks, DVM, Mount Forest, ON
Dongwan Yoo, Department of Pathobiology, University of Illinois at Urbana-Champaign, IL, USA

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.

Selected zoonotic pathogens and diseases identified at the AHL, 2009

Beverly McEwen, Durda Slavic, Davor Ojkic, Josepha DeLay, Hugh Cai, Margaret Stalker, Murray Hazlett, Marina Brash

Many new, emerging, and re-emerging diseases of people are caused by pathogens originating from animals, or are shared between people and animals. The AHL plays an important role in public health by identifying zoonotic pathogens (Tables 1 and 2). These are numerator data reliant upon submission biases to the diagnostic laboratory and cannot be regarded as population prevalence estimates. Monitoring programs are not included.

The zoonotic pathogens most frequently identified at the AHL since 1999 are *Leptospira* sp., *Salmonella* sp., *Streptococcus* sp., and *Cryptosporidium* sp. Occupational exposure to pigs and horses is a risk factor for *S. suis* and *S. zooepidemicus* infections. *Chlamydophila psittaci* was identified in 2 psittacine birds (included in the category 'Other'). The Ontario Health Promotion and Protection Act requires that any 'veterinarian who knows or suspects that a captive bird or birds or a poultry flock is infected with the agent of psittacosis or ornithosis shall notify the medical officer of health'.

Prior to 2008, the numbers of isolates were tabulated, however, due to the increasing number of tests for selected pathogens, the number of cases will now be documented. For data prior to 2008, please refer to previous editions of the AHL Newsletter. *AHL*

Table 1. Cases with selected zoonotic pathogens isolated and/or identified at the AHL, 2009

Agent	Bovine	Swine	Equine	Ovine	Caprine	Chicken	Turkey	Canine	Feline	Other	2009	2008
Blastomyces dermatitidis								10			**10**	Not done
Bordetella bronchiseptica		26	3					18	5	8	**60**	52
Campylobacter coli/ jejuni/ fetus subsp. fetus				2				6	1	5	**14**	14
Chlamydophila sp.				6	20					2	**28**	10
Clostridium difficile		13	8					2		1	**24**	25
Coxiella burnetii (Q fever)				2	7						**9**	15
Cryptococcus neoformans										1	**1**	Not done
Cryptosporidium sp.	115	1		3	5					4	**128**	144
Dermatophytes								2	3		**5**	Not done
*Eastern equine encephalitis virus**			11								**11**	12
Giardia sp.	14	1		1				35	4		**55**	56
Listeria monocytogenes	5			3	7			1		2	**18**	14
Methicillin-resistant *Staphylococcus aureus* (MRSA)		1	34					1			**36**	51
Mycobacterium tuberculosis											**0**	1
Rabies										8	**8**	4
Salmonella sp.	41	59	20	2	2	81	12	11	1	52	**281**	322
Streptobacillus moniliformis (rat bite fever)											**0**	1
Streptococcus suis		119		1							**120**	158
Streptococcus equisimilis		15	21					7			**43**	76
Streptococcus zooepidemicus	2	1	114								**117**	108
Toxoplasma sp.				10	8				1		**19**	8
West Nile virus			1							5	**6**	70
Yersinia sp.	1									2	**3**	6

Table 2. *Leptospira spp*. seropositive cases identified at AHL, 2009, microscopic agglutination test (MAT)

Leptospira* sp. serovar	Bovine	Swine	Equine	Canine	Other & not specified
L. autumnalis	5	3	15	32	19
L. bratislava	4	4	15	10	6
L. canicola	4	2	0	4	1
L. grippotyphosa	11	4	9	26	4
L. hardjo	8	1	0	0	1
L. icterohaemorrhagiae	18	5	11	12	2
L. pomona	28	5	17	29	3

* Some cases are from other provinces

AHL Lab Reports

RUMINANTS

Brachyspina syndrome in Holstein cattle – first identification in Ontario

Josepha DeLay, Jim Fairles, Bryan Hicks

Brachyspina syndrome describes the occurrence of multiple concurrent congenital anomalies involving primarily vertebrae with variable inclusion of heart, renal, mandibular, and gonadal defects. Affected fetuses are typically stillborn, often after a slightly prolonged gestation. The syndrome has recently been documented in Holstein cattle in Denmark and in the Netherlands. A hereditary basis for the syndrome has been hypothesized, likely with an autosomal recessive mode of inheritance, although the exact mutation responsible remains to be determined.

Affected fetuses are small for their gestational age and typically have long limbs with proportionately short bodies, due to severe malformation and fusion of cervical and thoracic vertebrae with shortening of the spine (hence 'brachyspina'). Brachygnathia is evident in some fetuses. Cardiac anomalies may be present and include ventricular septal defects and displacement of the base of the aorta, overriding the right ventricle (dextrorotation of the aorta). Renal dysplasia is common, and kidneys are typically very small and difficult to identify. Testicular and uterine aplasia have been reported, as has intestinal atresia.

We have recently identified lesions consistent with brachyspina syndrome in a stillborn Holstein fetus born to an Ontario-bred cow (Figure 1). Genetic testing is underway for further evaluation of the individual. This congenital defect is very similar to complex vertebral malformation (CVM), which has also been identified recently in Holsteins and has an autosomal recessive mode of inheritance. Fetuses with CVM also have shortened trunks with vertebral malformations and may have cardiac anomalies, however arthrogryposis is a consistent feature of CVM but is not seen with brachyspina syndrome. CVM fetuses typically approach normal weight for term fetuses (~25 kg), whereas fetuses with brachyspina syndrome are generally small (~10 kg). The specific mutation responsible for CVM has been identified and testing is available.

In an effort to eliminate these undesirable traits from the bovine genetic pool, **it is important that practitioners and producers recognize the external appearance of aborted fetuses with brachyspina syndrome and CVM**. These fetuses should be submitted to a diagnostic laboratory for confirmation of the diagnosis. It is equally important to notify breed associations of the occurrence of these hereditary conditions. *AHL*

Reference

Agerholm JS, Peperkamp K. Familial occurrence of Danish and Dutch cases of the bovine brachyspina syndrome. BMC Vet Res 2007;3:8.

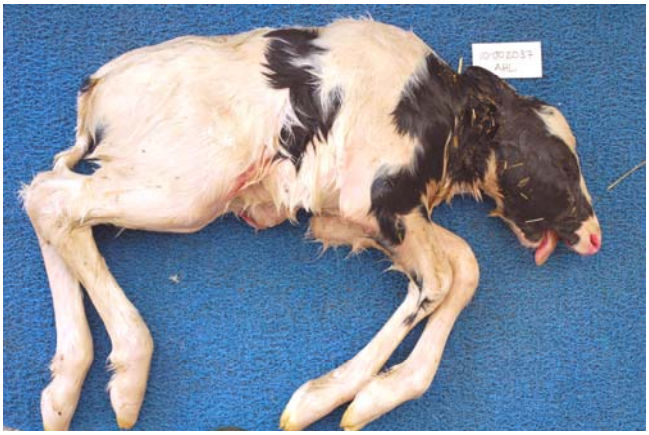

Figure 1. Stillborn Holstein fetus with brachyspina syndrome. Note the shortened trunk with hunched back (kyphosis) and long limbs. Brachygnathia is also evident. Limbs could be easily straightened, with no evidence of arthrogryposis.

Infectious causes of calf enteritis in necropsy specimens in 2008 and 2009

Maria Spinato, Beverly McEwen, Durda Slavic, Susy Carman, Hugh Cai

AHL diagnostic records for 2008 and 2009 were searched for infectious agents identified as common etiologic causes of enteritis in calves aged 1 day to 2 months (Table 1). The case definition was restricted to submissions in which both histopathology and microbiologic testing were performed. Cases comprised of feces or intestine submitted only for microbiologic analyses were excluded.

Not unexpectedly, 23% of calf enteritis cases were idiopathic. Factors that could explain this finding include:

- calf in later stage of disease and has cleared the infectious agent;
- prior antimicrobial therapy;
- inappropriate test or sample selection; or
- autolyzed tissue samples in which characteristic histologic lesions were not discernible.

Continued on page 5

Calf enteritis (continued from page 5)

Table 1. Summary of diagnoses of calf enteritis sampled at necropsy, AHL 2008 -2009.

Agent/condition	n	%
Cryptosporidium sp.	51	24%
Idiopathic	50	23%
Coccidiosis	19	9%
Compatible with viral etiology	18	8%
Salmonella spp.	17	8%
BVDV	11	5%
Coronavirus	11	5%
Escherichia coli	11	5%
Compatible with bacterial etiology	9	4%
Clostridium perfringens	8	4%
Rotavirus	7	3%
Giardia	1	0.5%
Trichuris	1	0.5%
Grand total	**214**	**100%**

For the years 2008 and 2009, the most frequently confirmed infectious cause of diarrhea in young calves was *Cryptosporidium* sp (24%). Bacteria were isolated in 17% of cases and lesions compatible with bacterial pathogens were present in an additional 4%. Similarly, viruses were identified in 14% of cases and lesions compatible with viral enteritis were present in 8%. In cases with diagnoses compatible with bacterial or viral pathogens, the appropriate ancillary tests were often not requested. Co-infections were diagnosed in 11 cases, and included a mixture of bacterial, viral and parasitic etiologies. However, diagnostic data obtained from necropsy submissions should not be interpreted as being representative of the most prevalent causes of calf enteritis on farm. Most calves experience mild, self-limiting diarrhea that responds satisfactorily to therapeutic intervention. In other outbreaks, an etiologic diagnosis may be obtained by fecal testing.

The relatively high proportion of cryptosporidiosis diagnoses may be partly due to the fact that this organism is easily identified histologically, even in autolyzed sections of intestine. Prophylactic and therapeutic options are limited for *Cryptosporidium* sp., and therefore, calves with this infection are more likely to die or to be submitted for post mortem examination. However, it has been reported that *Cryptosporidium* sp. may occur in 30-50% of diarrheic calves worldwide, and that case-control studies indicated a highly significant association with diarrhea. Therefore, despite the limitations of diagnostic data obtained from necropsy specimens, **cryptosporidiosis appears to have been an important and relatively common cause of enteritis in calves submitted to the AHL in 2008 and 2009.**

The diagnostic success rate in outbreaks of calf diarrhea can be increased by following the submission guidelines outlined in the December, 2008 AHL newsletter article, *Diagnostic approach to neonatal calf diarrhea*. *AHL*

Reference

Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary Medicine, 10th ed.. Saunders Elsevier, London, 2007: 847-851.

SWINE

Increase in proportion of PRRSV-positive cases identified in January 2010

Susy Carman, Li Ge, Beverly McEwen, Jim Fairles

Since the height of the PRRSV outbreak in Ontario swine in 2004, the "annual average" of PRRSV PCR-positive swine cases presented to the AHL has regularly declined from 51% in 2004 to 20% in 2009. The positive proportion rises seasonally in winter and declines in summer. For the last quarter of 2009, there was a slight increase in the proportion of PRRSV PCR-positive cases to 23%. This is followed by **a more definite increase in the proportion of PCR positive cases to 30% for the month of Jan 2010.** Serum or blood swab monitoring cases cannot be excluded, thus testing may represent enhanced monitoring of swine herds with ongoing infections, rather than all be new outbreaks.

In the late fall of 2009, we identified 7 cases from 6 farms with similar 98% sequence homology of ORF5. The strains caused abortion with subsequent severe respiratory disease in surviving piglets. **These strains had sequence-predicted RFLP type of 1-22-2.**

Gel-based ORF5 RFLP typing for PRRSV strains, as determined using Wesley's RFLP typing criteria, includes only 8 HincII restriction patterns. In the last few years, there has been significant genetic drift in the ORF5 gene sequence, with many strains now being reported to be "undetermined" at this restriction HincII site, when only gel-based typing is used. Although RFLP type names are a convenient way to track viruses, sequence homology provides a better comparison.

For comparison of PRRSV strains, we strongly recommend that you request ORF5 gene sequencing, which provides the % homology over the entire gene sequence, rather than gel-based ORF5 RFLP typing, which compares only a few nucleotides. The comparison of the % homology over the entire genome gives the best comparison.

When sequencing is requested, the AHL also provides the Minnesota based "sequence-predicted RFLP typing classification". This classification, based on the Wesley system, now extends the RFLP typing classification to include 3 MluI patterns, 39 HincII patterns, and 5 SacII restriction enzyme patterns. More patterns are being identified every month. These can only be determined following sequencing, and using the sequence to predict the RFLP type. *AHL*

AVIAN/FUR/EXOTIC SPECIES

Infection with a pathogenic *Turkey coronavirus* isolate negatively affects growth performance and intestinal morphology of young turkey poults in Canada.

Maged Gomaa, Dongwan Yoo, Davor Ojkic, John Barta (From Avian Pathology 2009;38:279-286.)

Turkey coronavirus (TCoV) is an important viral pathogen causing diarrhea of young turkey poults that is associated with sizeable economic losses for the turkey industry. A field isolate that was found to be free from turkey astrovirus and avian reovirus was used to experimentally infect turkey poults. Clinical signs and weight gain of poults during experimental infections were compared with age-matched, uninfected controls.

Poults infected at 2 days of age had 100% morbidity and 10% mortality, and birds infected at 28 days of age showed 75% morbidity and no mortality. Diarrhea was consistently seen in infected poults at 2 to 3 days post infection (dpi) with a duration of about 3 to 5 days. Mean body weights of birds infected at 2 or 28 days of age were significantly reduced compared with uninfected birds by 7 dpi and remained significantly lower for the duration of the study. At 44 days of age, poults infected at 2 or 28 days of age weighed only 68.1% or 77.7%, respectively, compared with uninfected turkeys of the same age on the same diet, a mean difference in body weights of 683 or 477 g, respectively. Infected birds had profound villus atrophy with some compensatory crypt hyperplasia at 5 to 7 dpi. Villus heights in the duodenum were significantly reduced at 7 dpi.

We were able to reproduce enteric disease using only a pathogenic field isolate (MG10) of TCoV that negatively affected growth performance and intestinal morphology of young turkey poults. *AHL*

Sudden death in rabbits

Marina Brash, Michael Taylor

Very few conditions cause sudden death in rabbits without premonitory signs. *Pasteurella multocida* infections may be expressed clinically as rhinitis, conjunctivitis, otitis media, abscesses, bronchopneumonia and pleuritis, or genital tract infections. Most rabbits with *Pasteurella multocida* infections will show some clinical signs including localized swellings, lethargy, sneezing, anorexia, nasal and/or ocular discharge.

The peracute form of pasteurellosis, which leads to acute sepsis may cause sudden death without any clinical signs. Often the history suggests that the rabbit had been normally active when last observed but was found dead a few hours later by the owner. This distressful situation frequently leads clients to seek assistance from their veterinarian to identify the cause of death. At necropsy, the lungs are usually congested and edematous, the spleen is enlarged and dark, and there may be purulent exudate in the nasal turbinates or within the tympanic bullae. The stomach typically contains feed, the small intestinal content is unremarkable, and normally formed fecal pellets are present in the descending colon and rectum. Bacterial culture of lung and spleen or liver reveals large numbers of *Pasteurella multocida*. Histological lesions are consistent with septicemia, with fibrinous thrombi within pulmonary capillaries and hepatic sinusoids, generalized tissue congestion and sometimes small intravascular colonies of short rod-shaped gram-negative bacteria.

At the AHL, a few cases of acute *P. multocida* septicemia are seen each year, and bacterial culture of affected tissues is needed to confirm the diagnosis.

The second cause of unexpected sudden mortality in pet rabbits is gastric dilation. At necropsy, a large, distended gas- and fluid-filled stomach, usually without volvulus, is the major finding. In the cases seen by one of the authors, obstruction to gastric outflow has not been a feature. Death is likely the result of pain of the markedly distended stomach as well as the pressure on the diaphragm with respiratory impairment.

Neither of these conditions is common, but when they do occur, the client will be shocked and upset and will likely contact their veterinarian to help provide some answers. If the necropsy is being conducted in-house, we recommend collection of tissues such as lung, liver and spleen and submit in addition to routine formalinized tissues with a note saying hold for further testing pending histology results or alternatively, let us know that these tissues have been frozen and retained at the clinic. If the histological lesions are suggestive of septicemia, bacterial culture can then be performed to determine the etiology.

Identification of either condition is possible using routine necropsy and ancillary testing and will help the owner deal with the sudden loss of a pet. The process can also foster an enhanced relationship with the veterinarian while also providing an opportunity for client education to help keep the other rabbits in the household healthy. *AHL*

HORSES

Ontario Racing Commission Death Registry: 2003-2009 necropsy summaries

Josepha DeLay

The Ontario Racing Commission Death Registry has been in place since 2003, and continues to provide excellent data regarding the causes of morbidity and mortality in racehorses in this province. Necropsy requests by the ORC have become more selective in the past 2 years, with a shift in emphasis to non-fracture cases with increased complexity. Summaries of necropsy submissions to the Animal Health Laboratory under this program and diagnoses for these cases are provided in the following tables. *AHL*

Table 1. Breed distribution of ORC Death Registry submissions to the AHL, 2003-2009

Breed/Year	Standardbred	Thoroughbred	Quarter Horse	Total
2003	67 (54%)	58 (46%)	0	**125**
2004	82 (58%)	60 (42%)	0	**142**
2005	59 (54%)	51 (46%)	0	**110**
2006	58 (54%)	47 (44%)	2 (2%)	**107**
2007	66 (54%)	53(43%)	3(3%)	**122**
2008	27 (53%)	24(47%)	0	**51**
2009	28 (62%)	16 (36%)	1 (2%)	**45**

Table 2. Necropsy diagnoses of ORC Death Registry submissions by body system, 2003-2009

Diagnosis by body system	2003	2004	2005	2006	2007	2008	2009
Fracture / limbs	53 (42%)	69 (49%)	48 (44%)	42 (39%)	54(44%)	16(31%)	**4 (9%)**
Fracture / other	10	4	7	13	10	5	**0**
Non-fracture muscu-loskeletal	8	6	6	8	6	5	**2**
Gastrointestinal	15	19	17	16	18	5	**4**
Respiratory	21	17	9	11	16	9	**21 (47%)**
Cardiovascular	5	6	5	5	2	4	**6**
Central nervous system	6	11	7	4	1	1	**2**
Integumentary	0	0	1	2	2	1	**1**
Renal	0	2	0	0	2	0	**1**
Hematopoeitic	2	1	1	0	0	0	**0**
Whole body conditions	1	7	5	2	9	0	**4**
Cause of death undeter-mined	4	0	4	4	2	5	**0**
Total	**125**	**142**	**110**	**107**	**122**	**51**	**45**

COMPANION ANIMALS

Cytology and surgical biopsy of canine mast cell tumors

Kristiina Ruotsalo, Josepha DeLay

The cytological features of canine mast cell tumors (MCTs) are usually straightforward, making cytology a useful and minimally invasive method for diagnosis. Typically, aspirates are highly cellular and consist of discrete individual round cells with abundant metachromatic intracytoplasmic granulation. Nuclear features are often obscured by this intense granulation. Numerous eosinophils are often present within these aspirates. A variable number of plump stromal cells and fibrillar, extracellular, eosinophilic material, consistent with collagenolytic debris, can be noted.

These described features are consistent with well differentiated mast cell tumors. Although tumor grading cannot be carried out on cytologic samples, **tumors can be loosely classified on the basis of their cytological appearance into well, moderately or poorly differentiated neoplasms by evaluating the degree of cytoplasmic granulation and nuclear atypia.** Cytoplasmic granulation is less intense and nuclear morphology is more variable (e.g., binucleation, prominent nucleoli, anisokaryosis) with less well differentiated tumors.

Another criterion for malignancy is the presence of metastatic disease. Often lymph nodes draining a tumor site are submitted for cytological evaluation of possible metastasis. This can be problematic if only low numbers of well-differentiated mast cells are found within the lymph node aspirate, as such cells can normally be present in lymph nodes in low numbers. A similar dilemma exists if splenic or hepatic aspirates are submitted for evaluation of systemic involvement and only low numbers of mast cells are evident. The identification of large numbers of mast cells at these extra-lesional sites, especially if only moderately or poorly differentiated in appearance, will aid in the cytological diagnosis of metastatic disease.

Current histologic grading schemes for MCTs are applicable to cutaneous tumors only and have not been thoroughly evaluated for tumors at other anatomic sites. Grading is based on a combination of cellular features and the extent and location of the tumor within the skin, and as such requires examination of a surgical biopsy. Classification is often straightforward for small, well-differentiated grade I tumors and poorly differentiated grade III tumors. However, **histologic features place the majority of canine MCTs in the grade II category, and prognosis among this group can be variable**. Recently, ancillary techniques including immunohistochemistry for evaluation of both intracellular KIT (c-kit gene product, also known as CD117) staining patterns and proliferation markers (PCNA, ki-67), and PCR for detection of c-kit mutations have been investigated as potential prognostic indices. Research is ongoing regarding the best use of these techniques in the reliable prognostication of MCTs. Early studies have shown that **mitotic index as assessed in routine histologic sections is a useful prognostic indicator and, in combination with traditional grading schemes, can provide more useful prognostic information for grade II tumors**. *AHL*

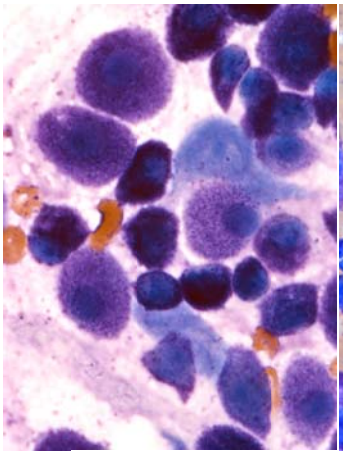

Figure 1. Cytology of a well-differentiated canine MCT.

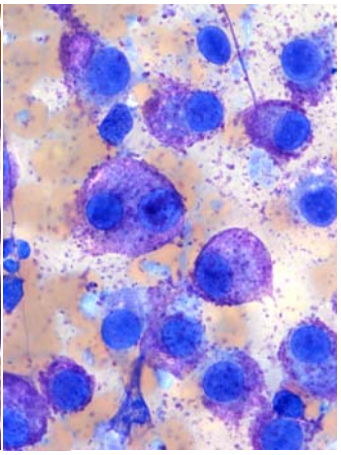

Figure 2. Cytology of a moderately well-differentiated canine MCT.

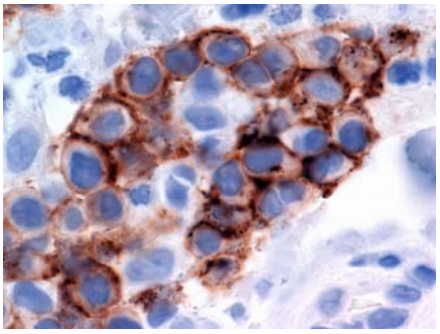

Figure 3. KIT (CD117) staining of neoplastic mast cells in a grade III cutaneous MCT. Cell membrane, cytoplasmic, and perinuclear staining patterns are evident.