

AKC CHF Health Conference, August 11-13, 2023  
Summaries of Selected Presentations (Elsa Sell)

Friday: Immunology and Microbiome

Saturday am: Prevention:

- [Dogs as sentinels of tick-borne pathogens](#)
- [Genetic tests for anesthetic drug sensitivity and post-op hemorrhage in sighthounds](#)
- [Approaches to genetic testing.](#) Asa Mays, DVM Award Recipient for 2023. Dr. Gary Johnson
- [Genetic study of risk factor for congenital idiopathic megaesophagus](#)

Saturday pm: Treatment

- [Use of microbes to fight obesity](#)
- [Gene therapy for the treatment of Doberman dilated cardiomyopathy](#)
- [Considerations and treatment of gallbladder mucocele](#)

Sunday: Progress Toward Cures

- [Role of DNA methylation in lymphoma](#)
- [Histotripsy treatment for sarcoma](#)
- [Defining margins in soft tissue tumors to aid surgery](#)
- [Hemangiosarcoma vaccine trial](#)

Friday

**Chronic Enteropathies** (3 presenters: Dr. Karin Allenspach, Iowa State U; Dr. Allison Manchester, Colorado State; Dr. Jenessa Winston, The Ohio State U). Chronic enteropathies are diagnosed after at least 3 weeks of symptoms and other problems are ruled out: i.e., parasites, cancer, extra-intestinal diseases (e.g., exocrine pancreatic insufficiency).

- Classification in order of diagnostic progression and increasing severity is:
  - Food Responsive (FRD): median age onset 3 yr, high percentage (60-70%) responded within 2 wks to an elimination diet; relapse rate 1.7 yr.
  - Antibiotic Responsive (ARD): median age onset 2 yr; required flagyl and if it is stopped the disease returns with a vengeance. Antibiotics or introducing abnormal bacteria (e.g., raw meat) may disturb the microbiome which leads to immune system disturbance; also the new bacteria may permanently replace the normal; has worst outcome; relapse rate 10 x/year.
  - Immunosuppressive/steroid Responsive (SRD): median age onset 6 yr; failed prior food and antibiotic treatment trials; relapse rate 4.7 yr
- The dog's signs can be useful to decide which diet to pair with the dog and also, the outcome; e.g., general attitude (reflect feeling bad), vomiting, bloating (select low fat), skin/GI signs, chronic otitis (allergic - change protein source), colitis symptoms (increase fiber content or profile). Even though 10-15% were non-responders, diet was the most powerful tool to manage chronic enteropathy in the others.
- Biopsy via endoscopy was not useful in deciding on treatment because the total number of inflammatory cells or the type of inflammatory cells are not different among the groups.

- A suggested approach to antibiotic use is none for mild disease with or without blood; none for moderate disease (use IV fluids for dehydration); use in severe disease with or without blood.

**Gut microbiome functions** as an organ to process all products of digestion which then go through the gut wall epithelial cells.

- The microbiome is 90% bacteria which differs with location (small intestine to colon) and environment (aerobic to anerobic).
- In disease states there is decrease in the normal thick mucus; that in turn allows epithelial cells to be more susceptible to bacteria.
- **Dysbiosis** in disease means altered community structure (different microbes present) and function (what metabolites are being produced).
- Gut microbiota can be identified by bacterial culture (least used because only detects 5-20%) or various molecular genetic techniques. **The dysbiosis index (DI)** is the only available test at this time for clinical use in dogs and cats (Texas A&M Vet). It is useful for chronic enteropathy. The canine test measures 7 different bacterial groups, including *Clostridium hiranosis*, a normal gut inhabitant which converts primary to secondary bile acids (these are antimicrobial). Higher DI indicates lower microbial diversity:
  - <0, normal; if individual bacterial groups are out of range, mild dysbiosis may be present
  - 0-2, mildly increased; some shift in diversity of microbiota
  - >2, consistent with shift in overall diversity of microbiota

[Back to page 1](#)

### Saturday morning - Prevention

**Tick-borne Pathogens** (bacteria, protozoa, viruses, others) (Dr. Barbara Quorllo, NC State U). Dogs are sentinels for these pathogens because of proximity to people, frequent exposure to tick habitats, similar susceptibility to tick-borne diseases, and they are screened more at routine wellness exams. Hosts are incidental (dog/people), reservoir (dogs), vector/reservoir (ticks). Hosts differ depending on year and location (state, county). The most shared diseases are Lyme, Borreliosis, Anaplasma, Ehrlichiosis, and Rocky Mountain Spotted Fever as determined by surveys and molecular diagnostics.

- Diagnosis depends on clinical suspicion and knowledge of regional disease. Lab diagnosis is made with titers or PCR testing for a specific pathogen, genus of pathogens, or serology (antibody detection). All diseases can be clinically significant so treatment and diagnosis are vital, often treatment simultaneous with diagnostic testing because of how rapidly the infectious agent causes progressive disease.
- The PCR technique on occasion identifies organisms with a pattern that doesn't match known rickettsii; research is ongoing to identify these organisms.
- [Back to page 1](#)

## Development of Genetic Tests (Anesthesia and delayed postop hemorrhage) for Sighthounds (Dr. Michael Court, Washington State U).

- **Anesthetic Drug Sensitivity** was discovered in greyhounds
  - With the observation of delayed recovery time after thiopental (a lipid soluble drug). It was discovered that thiopental sensitivity in sighthounds and to some degree in mixed-breeds was related to persisting higher drug levels.
  - In contrast propofol anesthetized dogs woke up sooner than when thiopental was used yet propofol levels declined more slowly in greyhounds than in mixed-breeds.
  - The slower decline in propofol levels was investigated and levels of a cytochrome P450 enzyme that metabolizes propofol was much lower in greyhounds. Research is ongoing to identify a gene mutation.
- **Delayed postoperative hemorrhage.** 28% of greyhounds showed delayed bleeding 48-72 hr after major surgery even though none needed transfusion. A condition called hyperfibrinolysis is present in cases of delayed bleeding. This means that even though blood initially forms a clot correctly, the clot later breaks down. The practical application is that some drugs can prevent hyperfibrinolysis post-op.
- The Scottish deerhound also has this condition. A survey of 269 dogs provided 8 delayed bleeding cases and 17 controls for study.
- Whole genome sequencing of these dogs identified a genetic mutation on the SerpinF2 gene (chromosome 9). This gene affects fibrinolysis by blocking a substance called antiplasmin. When the bleeders and controls were genotyped, the likelihood of bleeding was significantly higher in those with the 2 or 1 copies of the mutant gene than those with no copies (no bleeders). There is ongoing study of antiplasmin as a marker for hyperfibrinolysis in other breeds and the test (DEPOHGEN™ mutation) is available to owners through Washington State University.
- [Back to page 1](#)

**Asa Mays, DVM Award Recipient for 2023. Dr. Gary Johnson, U Missouri. Keynote Presentation.**

Dr. Johnson reviewed the process of identifying mutations related to canine diseases. There are now over 200 such tests and he contributed to a number of these discoveries. He also pioneered DNA banking for the parent clubs, leading to the OFA CHIC DNA repository, which is to be continued after his retirement with a substantial endowment from OFA. Samples in the DNA repository are available to research investigators on AKC CHF grants and others who qualify. This is an invaluable resource for canine research; owners of registered purebred dogs are encouraged to participate.

He reviewed how DNA can be multiplied with a process called polymerase chain reaction (PCR), how a thermostable DNA polymerase was found in the Mushroom hot spring in Yellowstone National Park. That enzyme resulted in a much larger supply of DNA used with the PCR process in developing genetic tests (e.g., canine von Willebrand Disease, protoporphyria in Limousin cattle, and neuronal ceroid-lipofuscinosis in English Setters.)

More recently, the PCR technology has been superseded by whole genome sequencing (WGS). The cost of WGS has diminished from \$3 billion for the human genome (1990-2003) to \$745 in 2023 when submitting 35-40 samples together.

Dr. Johnson reviewed his lab's more recent role in the process identifying the cell types involved in Canine Degenerative Myelopathy. He has been a most busy and productive investigator on behalf of purebred dogs.

[Back to page 1](#)

**Genomic Studies Reveal Risk Factors for Congenital Idiopathic Megaesophagus.** (Dr. Leigh Anne Clark, U of GA).

Megaesophagus is a motility disorder of the esophageal muscle. In the dog, food moves upwards in the esophagus to arrive at the stomach (in the human the movement is downwards). Megaesophagus is congenital in about 75% of cases and acquired in the others. Diagnosis is made with standard or barium swallow radiography.

- About 28% of cases are diagnosed in German Shepherd Dogs although it can occur in all breeds. This high incidence of GSD provides opportunity for genetic study. It is known in GSD that males are overrepresented and some populations like service dogs are more significantly affected. It is independent of body size when birth weight is controlled for.
- Ongoing research (Whole Genome Sequencing) is directed at identifying alleles (genes) on chromosome 12 that are associated with melanin-concentrating hormone receptor which is likely related to megaesophagus. They are testing samples from an affected service dog, a control service dog, a pet case, and a pet control. The study along with those in other institutions hopefully will result in a useful genetic test for breeders.
- [Back to page 1](#)

### Saturday afternoon - Treatment

**Harnessing the Power of Microbes to Fight Obesity: A First Look at the Canine SLIM Study.** (Dr. Jenessa Winston, The Ohio State U)

There is an obesity epidemic among companion animals (60% cats, 56% dogs) and obesity is the most common disorder in dogs.

- Among the causes
  - Animal (genetics/breed, neuter status, age)
  - Owners (diet, how fed, exercise, living environment, attitudes, owner age/size). Most vets believe the problem is due to owners.
  - Vets. A study learned that what is communicated during a veterinary visit may play a role. It was found that communication about nutritional history and body condition at vet visits to be brief (av 46 sec) and poor.

- Consequences of obesity include decreased life expectancy and quality of life, chronic inflammation, kidney dysfunction, lots of other system disorders - just as in people.
- Current treatment recommendations include feeding less calories (after a thorough nutritional assessment including snacks, treats, etc.) and more exercise (has to do with owner factors too). Owner compliance controls success.
- Dr. Winston has focused on dysbiosis as a possible way to reduce weight because dysbiosis has been documented in obese animals. That means the normal gut bacteria in the gut (bacteroides and a *Clostridium hiranosis*) are decreased and potentially pathogenic bacteria (staph, e coli, salmonella, pasturella) are increased. It is not known whether dysbiosis causes obesity or the reverse.
  - Their laboratory investigated efficacy of fecal transplant on weight reduction. Dogs in at least body condition 8 were followed for 24 wks after fecal transplant using a randomized control trial. The goal was to have 30 dogs but 23 were eliminated due to parasitic infection. Most lost 20% of weight. Dietary intake was adjusted for dogs who lost excess weight or were not losing enough weight. The study is ongoing.
- [Back to page 1](#)

### **Gene Therapy for the Treatment of Doberman Dilated Cardiomyopathy.** (Dr. Margaret Sleeper, U of FL)

The basic approaches to treating a heritable disease are

- Replacement for what is missing in the tissue (e.g., factor VIII in hemophilia)
- Local gene transfer which delivers high concentrations of therapeutic proteins to local tissue. This has been used in dogs and cats with lysosomal storage disease, hemophilia, retinal problems, and muscular dystrophy.
- The most commonly used substance to package a gene for transfer into tissue is viruses.

**Lysosomal storage disease (mucopolysaccharidosis VII)** causes myocardial disease from thickened a mitral valve by 6 mos. A study of 3 day old puppies (I did not get breed identify) injected the missing lysosomal enzyme with a retrovirus as the packaging agent. Treated pups had good musculoskeletal formation (and ability to walk), abnormal gait, and no cardiac disease. Untreated puppies had cardiac disease. Treated pups had relatively normal looking mitral valves on echo which was confirmed at autopsy.

The next task was to learn how to inject the enzyme directly into heart muscle. While several sites for injection did not work, endocardial delivery was efficient and produced good results with no mortality or secondary valvular insufficiency. The endocardium is the innermost layer of the heart, lining the chambers, valves and other structures.

**Duchenne Muscular Dystrophy in Golden Retrievers.** This disease is X-linked, has a cardiac component, and severity depends level of a protein called dystrophin. Treatment with a gene injection is difficult because the gene is too large to package with any available virus. An alternative approach was small RNA packaging called exon skipping that allows the body to make a shorter usable dystrophin. In golden retrievers the technique resulted in a 15-20%

production of dystrophin when the dose was high enough; there also was functional improvement.

**Dilated Cardiomyopathy.** Although this condition was called idiopathic earlier, the cause in certain breeds is now known to be a genetic mutation. It is the second most common heart disease in dogs, particularly larger dogs such as Dobermans (the highest %), Great Danes, Newfies. The condition causes ventricular arrhythmia (erratic heartbeats) and sudden death, or congestive heart failure. In Dobermans the lifespan is short with some pups dying as early as 3 months.

The research treatment protocol used multiple heart muscle injections with a low dose vector. Eleven have been treated so far with mean survival of 6.9 mos in 10 dogs; most causes of death were cardiac. The goal of future research is to try to initiate treatment before onset of heart failure.

[Back to page 1](#)

### **Evidence-based Approaches to Treatment of Dogs with Gallbladder Mucocele Formation.**

Dr. Jody Gookin, NC State U)

The normal gallbladder is thin and lined with clear mucus. With mucocele formation the mucus gradually becomes thick and dehydrated; eventually that causes rupture of the gallbladder. Mucus also obstructs flow of bile. Once there are signs of gallbladder illness, surgery is the only option and there is high mortality. Dogs who were asymptomatic before surgery had better outcome (4.7% mortality vs 17.4% in symptomatic). It is not known why the mucocele develops and there is currently no way to treat mucocele formation.

Breed predisposition exists so some genetics must be involved; 90% were purebred with a variety of breeds. It is generally a disease of older dogs and there can be associated endocrine/metabolic disorders such as hypothyroid, hyperadrenocorticism, hyperlipidemia, hypercholesterolemia.

One theory in recent years is that exposure to a toxicant (called xenobiotic) of some kind (diet or environmental) to mucocele development. Main target is the gallbladder. If the xenobiotic were an endocrine disruptor that would explain the high incidence of endocrinopathy. Toxicology research on endocrine disruptor exposure has shown dog gallbladder abnormalities that are similar to those in mucoceles.

A study in dogs with mucocele found occult hypothyroidism in 25% problem and in none of controls. Another study looked at metabolomics profiling of serum and bile and found much metabolic disruption with failure to clear metabolites (carbohydrates, lipids, amino acids). This is similar to metabolic syndrome in humans. Mucocele dogs were also deficient in a large number of cofactors, enzymes, and vitamins; these are important in metabolism including the type of mucus produced. The findings raise the question of whether correcting these metabolic deficiencies could affect mucocele formation in dogs.

Their ongoing study selected dogs either with an early diagnosis of mucocele (presurgery) or the owner opting to not do surgery in more advanced cases. They were tested for and then treated if there was concurrent illness (hypothyroid, Cushings, hyperlipidemia, proteinuria).

Followup was with quarterly ultrasound for one year. The goal is to determine if treatment of concurrent illness could delay, slow, or stop development of the mucocele.

Another ongoing study is designed to determine whether a xenobiotic substance is present in bile acids. This is important because bile is a major route for discarding water-insoluble (i.e., lipophilic) substances including xenobiotics. Abdominal fat was taken from 15 dogs with mucocele and 15 controls at surgery. Cells were extracted for tissue culture; these tissue culture cells formed mucus so they could be evaluated to screen different xenobiotic compounds for pathologic effects. The proteins in the thick and dense mucus of mucocele dogs was very similar to mucous found in human cystic fibrosis. To date, no gene similar to the cystic fibrosis gene has been found in the dogs; research is ongoing.

[Back to page 1](#)

### Sunday Morning - Progress Toward Cures

#### Diffuse Large B Cell Lymphoma of Golden Retrievers Has a Unique DNA Methylation Signature That is Highly Conserved. (Dr. Jeff Bryan, U of MO)

- **Definition of epigenetics:** all cells have the same DNA code, yet each cell performs its system's own function. Epigenetics governs which genes are to be turned on or off. This allows genes to function as intended or not. Cells stay within a system's lineage over time.
- **DNA methylation.** If the normal C-G (cytosine-guanine) sequence has a methyl group added to cytosine the sequence behaves differently; in other words, methylation can be a control mechanism within cells and it can result in problems
  - DNA methyltransferases maintain methylation pattern of parent strand after new DNA is synthesized so that every cell in lineage of parent cell is identical. Enzymes can add new methyl groups where cell need them depending on environmental stimuli to which the cell is responding. There are also enzymes that remove methyl groups.
- **Role of DNA methylation in cancer.** Environmental stressors can change DNA methylation patterns under chronic stress; for example, tobacco smoke. These changed patterns can be passed down from parents to children. Reproductive cells in embryos of those exposed to stressors can be passed on (epimutations); this may have some impact on cancer heritability in dogs separate from mendelian inheritance. Epigenetic changes may be modifiable and used to cure cancer.
  - DNA methylation patterns are heritable and generationally stable, modifiable through drugs and dietary manipulation. A gene can have either a lot or little of methylation. When there is little methylation or none, the tumor is more malignant. Methylation biomarkers exist in dogs who have and who have not yet developed lymphoma. There is need to understand what contributes to the variability of methylation and how that might be manipulated by diet/nutrition.
- In the Golden Retriever there are different types of B cell lymphoma. The aggressive form has only 1 cell type with overexpression of (i.e., more) methylation. Study is being done to identify potential future cases of cancer using methylation markers. Some normal dogs had high levels of markers - in 1 case, the dog developed

hemangiosarcoma; in another, the dog developed lymphoma; 2 other dogs have so far remained normal. The goal is to replicate finding in humans that the likelihood of detecting cancers is in 70-80% range, some accomplished with methylation detection techniques.

- So far it has been found that methylation biomarkers in dogs can separate lymphoma from all normal lymphocytes.
- [Back to page 1](#)

### **Histotripsy: A Novel Treatment for Osteosarcoma.** (Dr. Joanne Tuohy, Virginia-Maryland College of Vet Medicine)

Osteosarcoma is the most common bone tumor of dogs and it happens mostly in large dogs. This is also the most common tumor in children and adolescents. The disease affects similar locations, the cancer cells look the same, and there are similar gene mutations between the 2 species. Dogs share home environment and stressors.

Challenges of osteosarcoma treatment are extreme pain and metastatic disease which limits the ability to extend survival times. The approach to extreme pain has been limb salvage surgery or amputation; both are difficult procedures and avoided if possible.

**Histotripsy uses ultrasound which is non-thermal to ablate osteosarcoma.** It is a mechanical procedure focusing ultrasound waves into tissue. The procedure causes cell destruction. Another treatment to which there was no response is immunotherapy.

- One part of their research was on excised osteosarcoma tissues. After preparation, the tissue was treated with histotripsy. Normal bone was used for control. After histotripsy tumor cells disappeared.
- In a separate clinical trial, blood samples were taken for immune cell evaluation and after lithotripsy treatment (1, 3, or 5 days later) either amputation or standard chemotherapy was done.
  - Complications of the system used to deliver histotripsy were aspiration pneumonia and gastroesophageal intussusception.
  - Treatment effectiveness was measured with imaging and peripheral monocytes.
    - MRI was better than CT and showed high correlation in 7/8 patients.
    - Peripheral monocytes increased from baseline 1 and 5 days after treatment. Within the tumors there was monocyte infiltration. Mechanism for the immune response is subject of ongoing study.
  - With those findings, a new study has started in which tumor ablation is done with lithotripsy (no amputation). MRI will be done at intervals and fracture risk from weakened normal areas of bone is being assessed. The dogs remain in the study until owner chooses euthanasia.
- [Back to page 1](#)



**Improve Patient Outcome Looking at Margins of Soft Tissue Sarcoma or Mammary Tumors.**  
(Dr. Laura Selmick, The Ohio State U).

Improved technology is needed to assess tumor margins rapidly and accurately. Optical imaging at surgery called Optical Coherence Tomography (OCT) is one possible technique. In their study of observer accuracy, it was good in soft-tissue sarcoma, less good in mammary tumors. The latter perhaps because mammary tissue and tumor type was highly variable whereas that was not the case for soft-tissue sarcoma. An improved technique called polarization-sensitive OCT visualizes collagen with better contrast to identify tumor margin edge. This technique is beginning to be used in both human and canine tumors.

[Back to page 1](#)

**GD3 Nano-scaled Liposomal Cancer Vaccine Clinical Trial for Canine Hemangiosarcoma.**  
(Dr. Rowan Miller, U Florida)

This is an immunotherapy vaccine that used in osteocarcinoma and more recently for hemangiosarcoma. It is directed toward a substance called GD3 (and/or GD2) which is a ganglioside identical in all species and present in a number of tumors. It behaves like an antigen which immunotherapy can attack. The vaccine is prepared by adding GD3 (and later GD2) to liposome which combination causes a reaction at the tumor site; how is not known.

Their study in splenic hemangiosarcoma (with controls and randomization) is to evaluate response to vaccine with a placebo controlled, randomized, double-blind study (the gold standard technique). The nature of hemangiosarcoma presents difficulties for this research design because:

- The cancer usually presents as an emergency with the high initial costs (to owner) for bleeding control, splenectomy, staging) before a trial.
- That interferes with obtaining a good enough tissue sample to study GD3
- The primary tumor needs to be identified (since this one is for the spleen); is it spleen 50-65%, right atrium (3-25%) or liver (5-6%)?
- The tumor type needs to be no greater than II.
- So far 26 dogs were screened, 13 had hemangiosarcoma, 4 were enrolled (1 is alive, others deceased). The study will be unblinded at 12 cases.

Other complicating factors are: 1) expression of different levels of gangliosides (GD2 and GD3) in primary tumors (so a single vaccine dosage may not work), 2) immune cells cannot always get into the tumor, 3) different cell types found in hemangiosarcomas can affect response, and 4) the initial GD3 vaccine had to be modified to include GD2.

[Back to page 1](#)